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POT TESTS OF NEMATICIDES AGAINST POTATO-ROOT EELWORM

I. PILOT TEST AND METHODS

By B. G. PETERS

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(With 1 Text-figure)

Details are given of nematicidal tests, directed against the potato-root eelworm, in 8 l. glazed pots of infested soil. The soil was sampled a few weeks after injection to secure data on the kill achieved, by counting the eelworm larvae hatching in root diffusate. A potato tuber was then planted in each pot and yielded information on phytotoxic or soil-amendment effects. A second soil sample at the end of the season showed the degree to which the eelworm population had recovered after treatment. With only duplicate pots, and single batches of 100 cysts from each pot, clear-cut results were achieved with D-D mixture and ethylene dibromide. In the present pilot test, the data were handled by ordinary analysis of variance methods, by which the lowest significant percentage kill was 73 %, but the methods could be adapted for purposes of probit analysis.

INTRODUCTION

The potato-root eelworm (*Heterodera rostochiensis*) is a major source of economic loss in parts of north-west Europe and in Long Island, U.S.A. Because of the long survival in soil of the dead protective cyst and of its content of viable eggs, rotation with non-susceptible crops must be very protracted to achieve control. There is need for a good nematicide, whether fumigant or not; but the high rate of multiplication of survivors necessitates a high rate of kill by the nematicide or, alternatively, its use at frequent intervals. Taking economic factors into account, the best available nematicide against this parasite is probably D-D mixture, but Peters & Fenwick (1949) have shown that this can give disappointing results under field conditions in England.

The search for new nematicides goes on, but estimations of their efficacy are very expensive if they are used on a field scale or even in small plots. It is true that techniques of known accuracy are available for estimating the density of cysts per gramme of soil, and of eggs and larvae per cyst. But both of these estimations are subject to high variability. The number of eggs per cyst ranges from several hundred to zero, and there may be a high proportion of empty cysts; large numbers of cysts must therefore be used in each unit test. Cysts are seldom distributed at random in the soil of a field or plot; sampling of the unit area therefore needs to be extensive. Even then, samples taken from the same plot at different times are seldom comparable in terms of larval density. This latter difficulty can be overcome

by using pots of soil, since the soil of a pot can be thoroughly mixed before sampling, and the mass of soil used for the experiment can be mixed before filling the pots. Experience has shown that nematicides tend to give a higher kill in pots than when used at comparable rates in open ground, but pot tests may still be valuable for comparing different nematicides, and if one of them has been extensively used on a field scale the relative efficacies should be a rough guide to field use.

The present paper describes a pilot experiment using nematicides in pots of soil naturally infested with *H. rostochiensis*, and employing various criteria to measure efficacy. The objects were to establish a method and to assess the sensitivity of the criteria in revealing significant effects. This sensitivity can be increased by replication, but it was desired to see what could be done with true replication reduced to a minimum; pots were therefore treated alike in pairs. From previous experience it seemed likely that the method would not detect small nematocidal effects, but in practice it is the large effects which are of main interest. The suggested method is in effect a preliminary screening of nematicides to exclude those of low efficacy.

METHODS

Glazed cylindrical pots were used, of 17.5 cm. diameter and 37 cm. high, with a drainage tubulure at the base, and holding about 20 lb. of soil (8.5 l.); the exposed surface was 240 cm.². The soil, from an allotment at Rothamsted, was a medium loam containing about 1.3 cysts per g. with 80 eggs per cyst, or 100 eggs per g. of soil. A mass of the soil was sifted through a $\frac{1}{2}$ in. sieve, coned and quartered three times, and poured into thirty-two pots.

Injections

It was decided to inject two nematicides of known efficacy, Shell D-D (a mixture of 1 : 3-dichloropropylene and 1 : 2-dichloropropane, with some higher chlorides) and ethylene dibromide at 5 % (v/v) in solvent naphtha, and also two chlorophenol products. The latter were mistakenly used as fumigants before it was discovered that their vapour pressures were too low to justify this; they could satisfactorily be used only in the form of solutions or suspensions in a large volume of water. For the purposes of a pilot experiment there is some value in a large number of ineffective treatments, as will be seen, and the chlorophenol pots were therefore retained.

All but control pots were injected on 21 April by inserting a glass tube with an external cork stop at 15 cm. deep and a temporary glass rod to prevent the tube from filling with soil. The soil was at 17°C. Each nematicide was used at the rate of 1, 4 and 16 ml. per pot, with two pots at each rate and two uninjected controls. By way of sealing the surface against the escape of vapour, 100 ml. of tap water was sprinkled over each pot.

The chlorophenol products will be referred to as P and C, ethylene dibromide as E, and D-D mixture as D, with a stroke number to indicate dosage rate in ml. per pot, e.g. E/4.

The pots were left outside in a 4×8 block, in which positions were allocated by randomization. The intention was to sample them after 4 weeks, but rain made them so wet that sampling was deferred until 7 June (47 days).

Sampling and criteria

The soil from each pot was emptied into a large tray and thoroughly mixed, and a sample of about 300 g. was withdrawn for air-drying and cyst recovery. The dry soil was sifted (6 meshes per inch) and 200 g. washed in the Fenwick apparatus as described in Peters & Fenwick (1949, p. 367), after which the cysts were counted and stored. At sampling it was noted that the two E/16 pots smelled of naphtha, and all the injected D pots of D-D.

The soil was replaced in its pot, over a layer of pebbles for drainage, and a tuber was planted. Tubers were selected for even size (mean weight 87 ± 2.4 g.). Pots were replaced outside in the original random order and left throughout the summer until the haulms had died down.

Twice during the season, at 21 and 45 days after planting, the heights of the haulms were recorded. At 21 days the D/16 pots were showing a severe check but had largely recovered by 45 days. At the end of the season the soil in each pot was sampled as before for cyst recovery and the tubers were counted and weighed.

The author has shown elsewhere (Peters, 1948) that height of haulms and weight of tubers are fairly sensitive criteria of plant growth, and the object of both criteria was to detect phytotoxic or 'soil amendment' effects as a result of treatment. As before (Peters, 1948) the 'number of tubers' criterion proved insensitive.

In field trials with D-D, Peters & Fenwick (1949) used three sets of soil samples taken: before injection (*X*), 4 weeks after (*Y*), and at the end of the following potato season (*Z*). In view of the thorough mixing in the present pot test, the *X* samples were dispensed with, the *Y* samples from untreated controls supplying an estimate of the eelworm population before treatment. The *Y* and *Z* samples from each pot yielded a count of cysts per 200 g. and also a batch of 100 cysts, which were subjected to hatching in root diffusate as described before (Peters & Fenwick, 1949, p. 368). Two 1 ml. aliquots from the suspension of hatched larvae were counted in the counting slide described and figured by Goodey (1951, p. 10), thus giving an estimate of larvae per cyst. The product of (cysts per g. of soil) and (larvae per cyst) gives (larvae per g. of soil), which is taken as an estimate of the eelworm density of that soil. For both *Y* and *Z* samples there are, therefore, three criteria which may be symbolized c/g , l/c and l/g .

Data in this untransformed state are not suitable for analysis of variance, since the standard deviation is roughly proportional to the mean instead of being independent. I have shown (Peters, 1948, p. 126) that the logarithmic transformation is satisfactory, and this has been used for all eelworm criteria. $\log(l/g)$ has been taken as $\log(c/g) + \log(l/c)$.

Cysts from the *Y* and *Z* samples were exposed to root diffusate during 9 weeks

in the spring following treatment, the final counts being available over a year after the experiment started. If the Y and Z counts are to be in any way comparable, it is essential that the hatching of both should proceed as one operation, using the same root diffusate under the same conditions. In this pilot test it was desired to make the counts comparable, but for routine purposes the Y diffusate hatching could proceed within a few weeks of taking the samples.

The six eelworm criteria, analysed in logarithmic transformation, have been taken as giving the following information:

(a) $\log (c/g)_Y$ tests adequate mixing of the original bulk of infested soil; it is assumed (with some evidence) that the density of cysts is not affected, within a few weeks, by the nematicide used.

(b) $\log (l/c)_Y$ estimates the nematicidal effect (so also does $\log (l/g)_Y$, but, being compounded of two criteria, it has a relatively larger variance and is unnecessary provided that $\log (c/g)_Y$ shows no spurious significant treatment effects due to poor soil mixing).

(c) $\log (l/g)_Z$ estimates the net effect on the eelworm population of treatment plus a succeeding potato crop.

(d) In terms of larvae per g., the ratio Z/Y , given in transformation as

$$[\log (l/g)_Z - \log (l/g)_Y],$$

estimates the multiplication factor for those eelworms surviving treatment. Similarly, the factor for multiplication since the start of the experiment can be estimated in transformation as $[\log (l/g)_Z - K]$, where K is the mean $\log (l/g)_Y$ for the eight untreated control pots.

Statistical analysis

A standard 4×4 design (4 nematicides at 4 rates), with each of the sixteen treatments duplicated, would provide degrees of freedom as shown in parentheses in Table 1. But in the present case there is a dummy treatment effect; since the eight control pots are treated alike, there are only thirteen treatments. Putting T for a treatment (pair) total and X for the grand total, the sum of squares for treatments,

$$(\Sigma T^2/2 - X^2/32) \text{ with } 15 \text{ D.F.},$$

is too large, and the analysis should have the degrees of freedom shown in the table. In fact the computations were done in the standard form and the sum of squares for treatments corrected by subtracting

$$(\Sigma T_0^2/2 - \Sigma^2 T_0/8);$$

the interaction sum of squares becomes decreased, and that for error increased, by the same amount. (T_0 is the total for each pair of control pots.)

In order to convey through the eye a clear impression of the differences between treatments and their significance, the chart shown on p. 452 has been prepared.

For this purpose it has been necessary to retain the eelworm criteria in logarithmic form. Each point shown is the mean value for the two pots treated alike. The shorter 'critical range' line has the value ts (in the particular case of the difference between means of pairs, the standard error of this difference is numerically equal to the standard deviation, s ; this multiplied by t for 19 degrees of freedom is the critical difference, differences greater than which will be deemed significant at the 5 % level of probability). The chart can be used by marking off the limits of the critical range along the edge of a piece of paper, which is then slid over the line of means; any two means separated by more than this distance are judged to differ significantly.

TABLE 1. *Analysis of variance*

	Degrees of freedom	
	Full analysis	Adjusted
Between nematocides	(3)	3
Between rates	(3)	3
Interaction N.R.	(9)	6
Between treatments	(15)	12
Within treatments (error)	(16)	19
Total	(31)	31

RESULTS

The results are summarized as eleven sets of treatment means in Table 2, where the eelworm criteria have been retransformed; they are thus geometric means. The controls are here treated as a single group of eight pots, as in the analyses of variance. The latter for ten criteria are given in Table 3; the 11th criterion of Table 2 is not a separate distribution requiring analysis, being got from $(l/g)_Z$ divided by a constant, viz. the control mean for $(l/g)_F$. Treatment effects and their significance are shown by means of the chart; here the eight control values have been left in separate pairs to avoid the use of a second 'critical range' value for comparisons of two with eight pots.

Table 3 shows that, with the one exception of the criterion $\log (c/g)_Z$, the mean squares for nematocides and rates are not significantly greater than that for interaction; this can be ascribed to the diluting effect of the chlorophenol compounds having no nematocidal action, and to significantly large interactions (itself due to the use of two effective and two ineffective nematocides). However, in most cases variances from all sources are significantly greater than error, which justifies the comparison of treatments.

Plant criteria

It is clear from the chart that the significant height differences at day 21 are those of D/16 and E/16 with a possible slight effect in E/4. Phytotoxic effects were produced by these heavy doses even with an interval of 47 days between injection and planting. By the 45th day these plants had partially recovered and the analysis of variance shows no significant effects.

The weights of tubers show interesting differences. Thus, low yields are given by E/16 and D/16 showing a residual phytotoxicity, whereas the 1 and 4 ml. rates for both nematicides have produced better yields than the controls; E/4, in particular, shows a complete recovery from the slight early set-back. The effect of opposite results at different rates is that the general means for E and D do not differ significantly from the controls, and so (Table 3) the nematicides mean square does not exceed that of error. There is no explanation for the anomalous tuber weights in D/o.

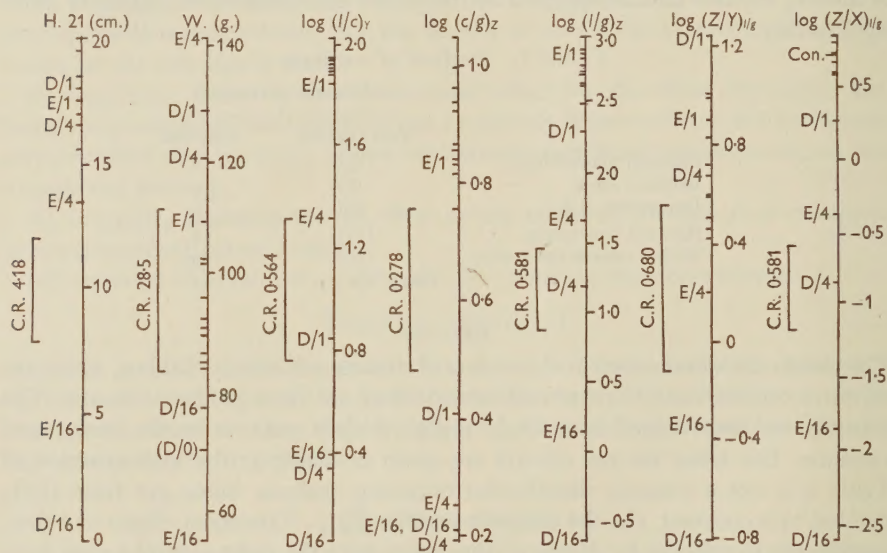


Fig. 1. Charts for finding significant treatment differences. The ineffective nematicides C and P, and the controls, are not labelled except in the last chart where 'Con.' marks the mean of eight control pots. C.R. = critical range (*ts*). H.21 = height of haulms (cm.) at 21st day. W = weight of crop (g.). $\log(l/c)_Y$ = mean log of larvae per cyst, Y samples. $\log(c/g)_Z$ = mean log of cysts per g. of soil, Z samples. $\log(l/g)_Z$ = mean log of larvae per g. of soil, Z samples. $\log(Z/Y)_{1/g}$ = larvae per g., mean log ratio of final to post-treatment counts. $\log(Z/X)_{1/g}$ = larvae per g., mean log ratio of final to original counts (estimated from controls).

Elkworm criteria

Analysis of $\log$ (cyst count) from soil samples (Y) taken 7 weeks after injection yields no significant effects; the soil mixing prior to filling the pots can therefore be taken as adequate. The criterion $\log$ (larvae per cyst) for these Y samples measures the immediate nematocidal effect. On the chart $\log(l/c)_Y$ shows that the rates 1, 4, 16 fall in the expected order for both E and D; E/1 reveals no effect and E/4 is significantly higher than E/16. D/1 is significantly higher than D/16, with D/4 about equal to E/16. The criterion $\log$ (larvae per gramme) for the same

TABLE 2. *Treatment means*

Treatment	Plant criteria			Eelworm criteria									
	Height (21) (cm.)	Height (45) (cm.)	Weight (g.)	Y Samples			Z Samples			Ratios (l/g)			
				c/g	l/c	l/g	c/g	l/c	l/g	Z/Y	Z/X		
P/1	20.0	44.5	87.0	1.27	80.4	102.3	8.51	47.3	403	3.94	4.01		
P/4	18.5	38.0	96.5	1.23	103.5	127.4	5.62	73.3	412	3.24	4.10		
P/16	17.5	39.5	84.5	0.87	87.1	75.9	7.24	102.3	741	9.77	7.38		
C/1	15.5	37.0	103.5	1.33	104.7	139.6	6.84	59.6	407	2.92	4.06		
C/4	19.5	39.5	93.0	1.41	86.1	121.6	6.46	75.0	484	3.98	4.82		
C/16	16.5	41.5	85.5	1.39	77.6	108.4	8.22	70.0	569	5.25	5.66		
E/1	17.5	40.0	110.0	1.20	70.0	84.1	6.68	101.2	676	8.04	6.73		
E/4	13.5	40.0	141.0	1.36	20.6	28.2	1.78	25.1	44.7	1.58	0.44		
E/16	4.5	30.5	55.0	1.26	2.4	3.1	1.60	0.9	1.4	0.47	0.01		
D/1	18.0	37.0	129.0	1.57	6.8	10.7	2.54	74.1	188	17.6	1.88		
D/4	16.5	41.5	120.5	1.50	2.1	3.2	1.55	9.6	15.0	4.68	0.15		
D/16	0.5	32.5	78.0	1.35	1.1	1.5	1.60	0.1	0.2	0.16	0.002		
Controls	18.1	42.4	88.6	1.26	80.2	100.9	9.12	59.7	544	5.4	5.4		

TABLE 3. *Analyses of variance: mean squares*

	Criteria ...	D.F.	H.21	H.45	W	log (c/g) _Y	log (l/c) _Y	log (l/g) _Y	log (c/g) _Z	log (l/c) _Z	log (l/g) _Z	log (Z/Y)
Nematicides	3	57.95 ^e		35.13	175.7	0.01803	2.4752 ^e	2.2701 ^e	0.4519 ⁱ	1.5228 ^e	3.6233 ^e	0.3233
Rates	3	125.78 ^e		54.87	2331.3 ^e	0.00560	1.0245 ^e	1.0790 ^e	0.3550 ^e	2.0195 ^e	3.4243 ^e	0.7065 ^e
Interaction	6	54.36 ^e		12.33	920.5 ^e	0.00360	0.6495 ^e	0.5880 ^e	0.0903 ^e	1.4269 ^e	2.0274 ^e	0.7813 ^e
Treatments	12	73.11 ^e		28.67	1087.0 ^e	0.007708	1.1997 ^e	1.1313 ^e	0.2469 ^e	1.5907 ^e	2.7756 ^e	0.6481 ^e
Error	19	3.994		32.52	185.2	0.007068	0.07272	0.07074	0.01764	0.06057	0.67718	0.10540

Note. *i*, interaction, or *e*, error, significantly exceeded at the 5% probability level.

samples gives similar results but with slightly less precision. It is of interest that the criterion for cyst count in the final Z samples, $\log (c/g)_Z$, would lead to the same general conclusions since the pots $E/4$, $E/16$, $D/1$, $D/4$ and $D/16$ all have significantly fewer cysts than the remainder, though there are no significant differences within these five treatments.

The criterion $\log (l/g)_Z$ measures the population of eelworm larvae after injection followed by a potato crop. In arithmetic form the range shown on the chart would be a very long one, extending from nearly 1000 l/g . for $P/0$ down to 0.2 l/g . for $D/16$. The treatment effects shown in the Y samples are still clearly present, with $D/1 < D/4 < D/16$ and $E/0 < E/4 < E/16$, but $D/1$ has shown a recovery of the population to a level not distinguishable from that of the controls. Zero on this scale represents 1 l/g .

The rate of recovery after treatment is a factor of some importance, and is measured by the ratio of $(l/g)_Z$ to $(l/g)_Y$, estimated in logarithmic form as the difference of their logarithms and shown on the chart as $\log (Z/Y)_{l/g}$. Zero on this scale represents no change in population between Y and Z and is the value that might be expected for an efficient nematicide. The chart will show that $D/4$, $E/4$ and $E/16$ do not differ from zero significantly, but $D/1$ and $E/1$ are significantly greater than zero with $D/1$ greater than some of the controls. The suggestion here is that the low rate of D-D represented by $D/1$ leads to an accelerated multiplication of eelworms, while $E/1$ and $D/4$ have multiplied (from different starting levels) at about the same rate as the controls. The case of $D/16$ is of interest, since it is significantly lower than zero, and this can only mean that the lethal effects of D-D have continued beyond the 7-week period between injection and sampling. This is in accordance with the observed phytotoxic effects, which belong to the same period. This being so, it can be concluded that a similar but slighter effect is shown by $E/16$. The discrepant behaviour of low and high rates in D and E has again led to a non-significant mean square for nematicides in Table 3.

The net change in population from before injection (X) to the final Z samples can be roughly estimated by taking $(l/g)_Y$ for the eight control pots as representing the population before treatment. If the logarithm of this value is subtracted, as a constant, from $\log (l/g)_Z$ the result will be the logarithm of the ratio (Z/X) , shown on the chart as $\log (Z/X)_{l/g}$. This line is equivalent to that of $\log (l/g)_Z$ with a different scale and the controls grouped. Zero on this scale represents no change in eelworm population between X and Z (or, if any change due to injection, then it has been nullified by the subsequent multiplication of surviving larvae). It will be seen that there has been a slight net increase in $D/1$, with significant net reductions in $D/4$, $D/16$ and $E/16$ and a slight reduction in $E/4$.

It will be seen from the various criteria on the chart that the values for the controls and for the ineffective C and P treatments lie together in a group which is reasonably compact as measured by the critical range, while the significant E and D values stand out clearly. The eelworm data, handled in logarithmic transformation by

analysis of variance, have yielded a number of valuable conclusions in spite of severe limitations on replication in the technique employed. To this extent the technique has justified itself for use in routine pot tests of nematocides. The fundamental criterion for this purpose is larvae per cyst in the *Y* samples, and these data can yield an estimate of the proportion of eelworms killed.

Percentage kill

For any given treatment (*t*), the ratio of $(l/c)_t$ to the corresponding count for the eight control pots is an estimate of the proportion of eelworms surviving the treatment: $p = (l/c)_t / (l/c)_0$. The proportion killed is then $q = (1 - p)$, and 100 times this value gives the percentage kills of Table 4. Similar results can be got from

$$100 p = \text{antilog} [2 + \log (l/c)_t - \log (l/c)_0],$$

but they are not identical because logs of counts are taken for each individual pot and the mean of the logs is not the log of the mean. It will be seen that the kills from E/16 and from all the D pots are around 90% or more; the low kill in E/1 (11.4%) is not significant as judged by the chart.

TABLE 4. *Percentage kill*

100{1 - [(l/c) _t /(l/c) ₀]}			
(Values in parentheses from logarithmic data.)			
E/1	11.4 % (12.7)	D/1	89.6 % (91.5)
E/4	73.8 % (74.2)	D/4	96.8 % (97.3)
E/16	97.0 % (97.0)	D/16	96.7 % (98.6)

In logarithmic transformation the mean of the controls is $C = 1.9038$ and the critical range is $R = 0.5645$. The smallest significant difference is $(C - R) = 1.3393$. The corresponding antilogarithms are for C , 80.13 *l/c* and for $(C - R)$, 21.84 *l/c*, the latter being 27.26 % of the former. This is the critical percentage of survivors, representing a critical kill of 72.74 %. Thus, the technique is not able to establish as significant any kill below 72.7 %. By this test the value of $\log (l/c)_t$ for E/4 is just significantly less than that of the controls (73.8 % greater than 0 %), as the chart confirms. In terms of percentage kill the technique is not very sensitive.

On the logarithmic scale used, an interval of two critical ranges ($C - 2R$) corresponds to a 92.6 % kill, $(C - 3R)$ to 98.0 %, and $(C - 4R)$ to 99.3 %, so that a series of nematocidal rates giving these kills would differ significantly. There is no theoretical limit to the number of multiples of R lying between 0 and 100 %, but a physical limit is set by the counting technique. The lowest possible count (1 larva in 2 ml. from 50 ml., based on 100 cysts) is 0.25 *l/c* corresponding to a 99.7 % kill, or just over 4*R* from zero.

In the purely practical sphere one is little interested in nematocides giving a kill lower than 75 %, and it is an advantage that the technique can discriminate more finely the higher the kill. For purely experimental purposes, however, it is

unfortunate that kills below 73 % do not differ significantly from zero, data like the E/1 kill of 11.4 % being valueless.

Had there been a sufficient number of dosage rates giving kills well spread over the possible range, the method of probit analysis would have been applicable, and values like 11.4 % for E/1 would be given due weight. Three rates are insufficient for this purpose, and the three points for E on a (probit $\times$ log dosage) plot are not in a straight line, but they suggest about 2.6 ml. per pot as the L.D. 50. The D values do not warrant extrapolation from 90 to 50 %.

Injection rates

For a variety of reasons, experiments in pots give only the slenderest guide to what can be expected on a field scale. But it may be worth recording that, based on the surface area of the cylindrical pots used, 1 ml. per pot is equivalent to 37.0 gal. per acre (416 l. per ha.), so that the equivalent doses of D-D and of 5 % (v/v) ethylene dibromide were 37, 148 and 592 gal./acre. The lowest rate of D-D used is equivalent roughly to 440 lb./acre, and is to that extent comparable with the 400 lb./acre dose used by Peters & Fenwick (1949) in field trials, where the best results at this rate (Gamlingay and Goole, Table 5 on p. 375) gave a kill of 50 %, compared with 90 % in the present pot test. Probably the greatest single factor favouring the pot test is the fine tilth of the soil combined with its freedom from plant residues.

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THE BIO-ASSAY OF POTATO-ROOT DIFFUSATE

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(With 5 Text-figures)

The effect of diluting potato-root diffusate is investigated. It is found that for any given sample the hatching ability is inversely proportional to the logarithm of its dilution and there is, moreover, for each sample a 'threshold' value of dilution beyond which it is inactive. The slope of dilution curves for different diffusates are all parallel. Use is made of these relationships in estimating the strength of any given sample.

When potatoes are grown in soil infested with potato-root eelworm, *Heterodera rostochiensis*, chemical substances produced by the growing roots stimulate the larvae to emerge from the cysts into the soil in search of a host. It is important that this fundamental aspect of the life cycle of the parasite should be studied in detail. There are, however, a number of factors which render the conduct of carefully controlled experiments difficult; for example, the number of eggs per cyst is very variable, the standard deviation for single cysts being of the same order as the mean (Fenwick, 1949). This error can be reduced by using batches of 100 or more cysts in four- or fivefold replication. Cysts from different sources also differ considerably from one another in their response to hatching stimulus, so that if a series of experiments be carried out on cysts from a single source the results obtained are applicable only to that particular stock. To obtain results of general application, it is necessary to use cysts from a number of different sources in a properly designed experiment so that one can analyse statistically the main effects, as well as differences between the different batches of cysts. Another variable factor is root diffusate which differs greatly in strength from one sample to another, since the method used for its collection does not lend itself to rigid control. Its rate of production may vary with age and variety of plant, climatic conditions, etc.; these variables can be partially overcome by bulking all leachings from a single batch of plants and storing the mixture at 35° F., at which temperature it retains its activity up to two years. It is thus possible to obtain a relatively constant diffusate for the conduct of a series of experiments and valid comparisons can be made within the series, but no absolute standards can be applied, nor is it at present possible to measure such phenomena as rate of breakdown of diffusate.

Various attempts have been made to standardize root diffusate. Picric acid at a concentration of 0.01 % can induce larval emergence (Fenwick, 1943), but attempts to relate its action to that of potato-root diffusate have been unsuccessful. Calam, Todd & Waring (1949) report that anhydrotetrone acid at concentrations

of about 1/2000 can act as a hatching agent, but experiments carried out by the present author indicate that it is nearly as inconstant from sample to sample as is the natural root diffusate. It works under different conditions from potato-root diffusate, and its dilution curve bears little if any relation to that of the latter. Ellenby (1944) sought to standardize potato-root diffusate by measuring the oxygen consumption of the roots which produced it; the method depends on the unproven assumption that respiration and root diffusate production, being different aspects of root metabolism, vary directly with one another. Since there is no experimental evidence for postulating this quantitative relationship it can only be considered as a rough qualitative test; moreover, it is useless for standardizing a sample of root diffusate in the absence of the plant from which it was taken. The present paper reports an investigation into the action of graded dilutions of samples of the natural product on cysts from different sources. It is suggested that the behaviour of these graded dilutions furnishes a satisfactory basis for the bio-assay of any given sample of root diffusate.

TECHNIQUE

In all the experiments described, cysts were set up in batches of at least one hundred in solid watch-glasses and presoaked for 2 or 3 weeks at a temperature of 24° C. The water was then removed and replaced by the appropriate sample of root diffusate. Tests were carried on either until all hatching had ceased, when the liberated larvae were estimated by dilution, or until the point at which the final hatch could be forecast by the method previously described by the author (Fenwick, 1951*b*). Data given here are total numbers of larvae in 1 c.c. samples and not the total larval emergence during the experiment.

PROCEDURE

Experiments were designed to investigate the following points:

- (a) The form of curve obtained when the hatch from a series of dilutions of root diffusate was plotted against dilution.
- (b) The changes in the curve resulting from the use of cysts from different sources.
- (c) Changes in the curve resulting from the use of similar dilutions of different samples of diffusate on a given cyst type.

(a) The effect of dilution of a single sample of diffusate

In a previous paper (Fenwick, 1951*a*), the author has shown that if a sample of root diffusate is successively diluted in a geometric series, and replicate batches of cysts are exposed to the action of each dilution, then if the number of larvae emerging are plotted against dilution, a straight line is obtained. To verify this record, a sample of diffusate was diluted with tap water, the dilutions chosen being 1/1, 1/4, 1/16, 1/64, 1/256, 1/1024 and 1/4096. Batches of cysts in fivefold replication were exposed to the action of each dilution, and the number of emerging

larvae per 1 c.c. of diluted suspension recorded. The total count per c.c. for each set of five batches was then plotted against dilution and the results are set out in Fig. 1. The hatch in pure water was also estimated and is represented on the graph by the line *AB*. It will be seen that for dilutions of pure diffusate ranging from 1/1 to 1/256 the points are in a more or less straight line. The regression line shown

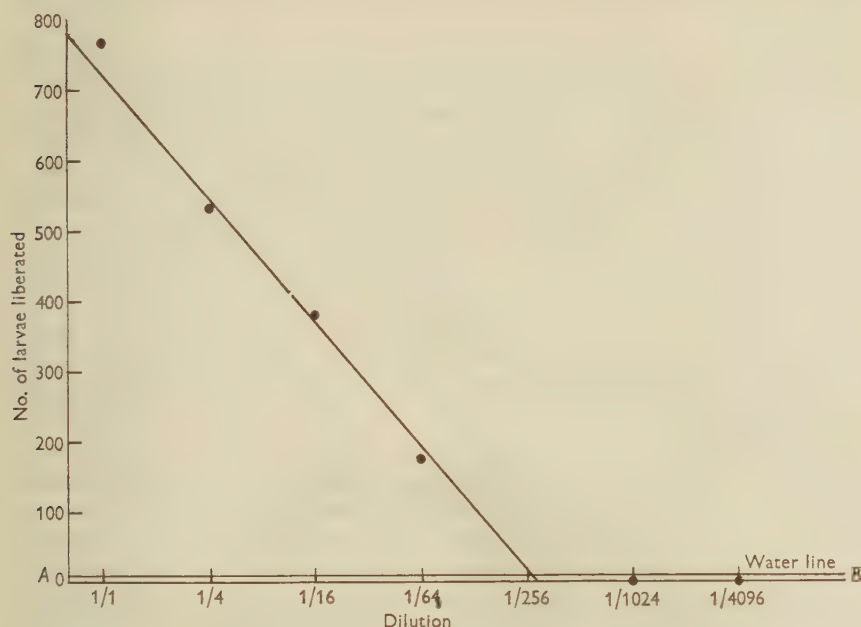


Fig. 1. The effect of dilution of potato-root diffusate on its activity.

TABLE 1. *Goodness-of-fit test for data of Fig. 1*

	Sums of squares	Degrees of freedom	Variance	Variance ratio
Error	6,144	15	410 (V_1)	—
Dilutions	84,703	4	21,176	—
Regressions	84,272	1	84,272	—
Residuals	431	3	143 (V_3)	$V_3/V_1=0.35$

in the graph was fitted to the points; a goodness-of-fit test was applied to it, and no significant departure from linearity was detected, the analysis of variance arising from this test being given in Table 1. Numerous repetitions of this experiment using different samples of diffusate on different samples of cysts confirmed this linear relationship. It therefore appears reasonable to conclude that the number of larvae hatching from cysts exposed to a single dilution of root diffusate is inversely proportional to the degree of dilution expressed logarithmically, i.e. hatch is proportional to the log-concentration.

(b) *The changes in the dilution curve resulting from the use of different cyst populations*

Having established the linear form of the hatching curve obtained by treating batches of cysts from a given source with a series of dilutions of a single sample of diffusate, experiments were conducted in which replicate batches of cysts from different sources were exposed to the action of a graded series of diffusates from a single stock.

To this end a single sample of diffusate was diluted with tap water in the proportions 1/1, 1/4, 1/16, 1/64, 1/256, 1/1024, 1/4096 and 1/16384. Four batches of 100 cysts each from Irlam, Gamlingay, Wainfleet and Sandy were exposed to the action of each dilution. Larval emergence from each cyst population in each dilution of diffusate was estimated and plotted against degree of dilution. Regression lines were fitted to the data for each cyst population and are seen in Fig. 2. Since each population exhibited a different degree of spontaneous hatching in water, and since four different water lines would be very confusing on a single graph, each curve in Fig. 2 has been depressed by an amount equivalent to the level of hatch in water so that the water lines in the data all correspond to the X -axis. Tests showed that no curve departed significantly from linearity. Table 2 gives data for estimates of the mean square from an analysis of variance arising from the goodness-of-fit test.

It will be seen that the four regression lines of Fig. 2 vary considerably in slope but appear to intersect the X -axis (which now represents the water line) at more or less the same point. The data from which the curves were computed were analysed and the coded value of dilution at which each curve cuts the X -axis (x_0) was calculated together with its respective standard error. The results are set out in Table 3; none of the values differ significantly from one another. Repetition of this experiment with other samples of diffusate and other cysts confirmed this result. It is therefore reasonable to conclude that the value of dilution corresponding to the intersection of the dilution curve with the water line has an absolute significance irrespective of the cysts used and characteristic of the diffusate in question. On the basis of the linear relationship between larval emergence and log dilution, and the common intersection point for different cyst populations in a single series of dilutions, it is possible to postulate a 'threshold value' of concentration below which any root diffusate is ineffective and above which its activity is proportional to the logarithm of its concentration. The 'threshold' is independent of cyst type and characteristic of the root diffusate.

(c) *Changes in the dilution curve resulting from the use of different samples of root diffusate*

As no two samples of root diffusate have the same hatching activity, experiments to investigate differences in the dilution curves of different diffusates were carried out. Four different diffusates were progressively diluted in the proportions 1/1, 1/8, 1/64, 1/512. Batches of 100 cysts in quintuple replication were exposed

to the action of each dilution and total larval emergence estimated. Computed curves showing the effect of dilution of each sample on larval emergence are given in Fig. 3. Goodness-of-fit tests showed no significant departure from linearity. As

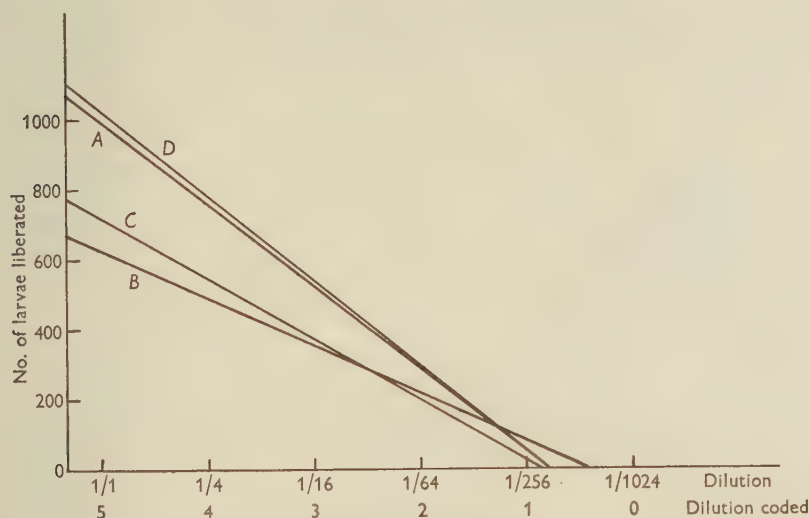


Fig. 2. Effect of exposing different cyst types *A-D* to the action of a graded series of dilutions of potato-root diffusate

TABLE 2. *Goodness-of-fit test for data of Fig. 2*

	Degrees of freedom	Mean square cyst type			
		Irlam	Gamlingay	Wainfleet	Sandy
Error (V_E)	15	3,420	574	410	1,396
Dilutions (V_D)	4	40,504	11,499	21,176	35,534
Regression (V_R)	1	157,252	45,428	84,272	140,185
Residuals (V_r)	3	1,589	1,897	110	650
V_r/V_E	—	0.46	3.28	0.27	0.47

TABLE 3. *Comparison of calculated estimates of x_0 for lines *A-D* in Fig. 2*

	x_0	V_{x_0}	S.E. $_{x_0}$
<i>A</i>	0.79	0.157	0.396
<i>B</i>	0.44	0.108	0.329
<i>C</i>	0.96	0.031	0.176
<i>D</i>	0.79	0.075	0.275

Maximum difference in $x_0 = 0.52$ for *B* and *C*.

S.E. of difference = 0.37.

$t = 1.45$.

all the cysts used in this sample were from a single stock the level of hatch in water is common to all four curves and can be ignored. It will be seen that the four curves vary in position, but their slopes approximate very closely to one another.

The data from which these curves were computed were analysed and the standard error of each value of the regression coefficient computed. The results are set out in Table 4, and it will be seen that the maximum difference in estimated values of b is only 1.74 times the value of its standard error, which suggests that the differences in the four estimates of b are not significant. This experiment has been

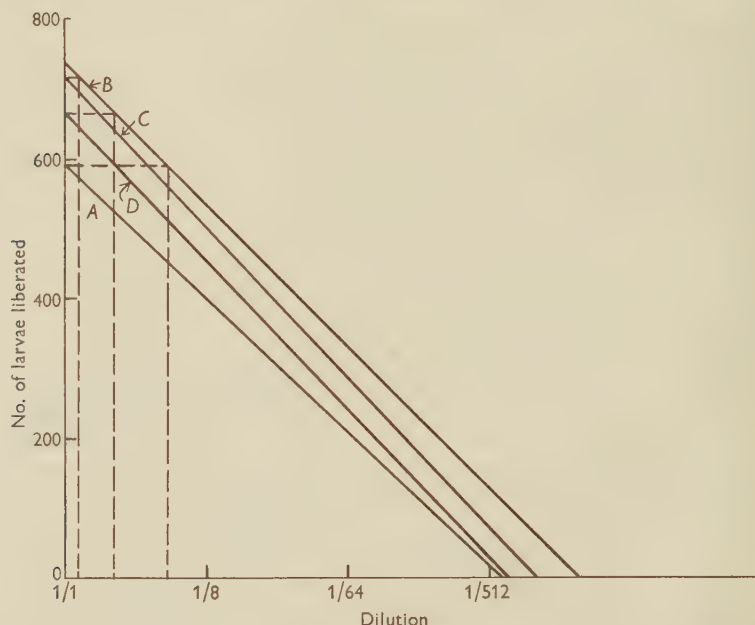


Fig. 3. Effect of graded solutions of different diffusates on larval emergence.

TABLE 4. Comparison of regression lines of Fig. 3

	b	V_b	S.E. _{b}
A	189.5	135.9	11.6
B	198.7	141.6	12.0
C	217.2	135.9	11.6
D	214.2	135.9	11.6

Maximum difference in $b = 27.7$ for C and A.

S.E. of difference for A and C = 16.5.

$t = 1.74$.

repeated with other samples of diffusate with similar results. It appears reasonable to conclude that when cysts from a given source are exposed to the action of similarly graded series of different samples of diffusate the dilution curves obtained are parallel to one another, the only differences in the curves being in position, i.e. in the point of intersection of each curve with the water line (x_0). It may be suggested therefore that the differences in position of these lines are reflexions of

differences of concentration of the active factor in the original undiluted diffusates. For example, diffusate *A* in its original form was weaker than *B* and was equivalent in strength to *B* at a dilution represented by the point *X*; undiluted *D* corresponds to *B* at a dilution *Y*, etc. It is thus possible to express the strength of any single diffusate in terms of any other tested.

Summarizing the results so far presented it is possible to put forward the following conclusions.

(a) The activities of different dilutions of a single diffusate on cysts from a given source are proportional to the logarithm of their concentrations.

(b) Each diffusate has a 'threshold value' of dilution beyond which it is inactive. This threshold is independent of cyst type and is characteristic of each diffusate.

(c) The dilution curves of several diffusates acting on cysts from a given source are parallel, and this parallelism enables one to express the concentration of one diffusate in terms of any other.

The bio-assay of any sample of diffusate can now be satisfactorily carried out starting from these basic principles. The threshold value of dilution forms a convenient reference point for any given sample—characteristic for that sample and independent of the cysts used. Since the chemical composition of diffusate is unknown, it is impossible to ascribe to this threshold value any absolute standard such as mg./c.c. and some arbitrary measure must be chosen. It is suggested that to a root diffusate diluted to its threshold, a value of one arbitrary unit be ascribed. Knowing the degree of dilution needed to attain this 'threshold value' it is possible to calculate the concentration of the original undiluted sample in arbitrary units. If the curve cuts the water line at a dilution of 1/1000, then the original strength of the diffusate was 1000 arbitrary units—a direct arithmetic measure of concentration. Since the dilution scale is logarithmic, interpolation is difficult, and it is proposed that instead of an arithmetic index of concentration, a logarithmic index be used. The term L.A. value denoting log-activity is proposed for this purpose, where the L.A. value is the logarithm of concentration in arbitrary units. The L.A. value will then be directly proportional to the hatching power of a sample on a given cyst type. The parallelism of dilution curves for different diffusates makes it possible to express the concentration of any one in terms of that of any other tested on cysts from the same stock at any given time. Diffusates of similar strengths in arbitrary units will have similar activities. If the L.A. value of each is calculated then the ratio of their L.A. values will be a measure of their relative activities.

THE DERIVATION OF L.A. VALUES

The derivation of an L.A. value for a given diffusate is a relatively simple matter. The given sample is put up in a graded geometric series of dilutions; a factor of 4 is convenient, and in this case the dilutions will be 1/1, 1/4, 1/16, 1/64, etc. Replicate batches of at least 100 cysts in fivefold replication are exposed to the action of each dilution, and larval emergence in each dilution estimated either after

allowing hatching to proceed to completion or by the shortened method of Fenwick (1951). Larval emergence is plotted against log dilution (Fig. 4), and a straight regression line is fitted to the data either by eye or calculation. The degree of spontaneous hatch in water is recorded on the graph by a line AB . The point of intersection O of the regression line with AB can now be ascertained and a perpendicular OX dropped from this point. This line will correspond to a concentration of 1 arbitrary unit. A new scale of arbitrary units CD can now be drawn with C as the origin and using the same interval as the original scale of concentration.

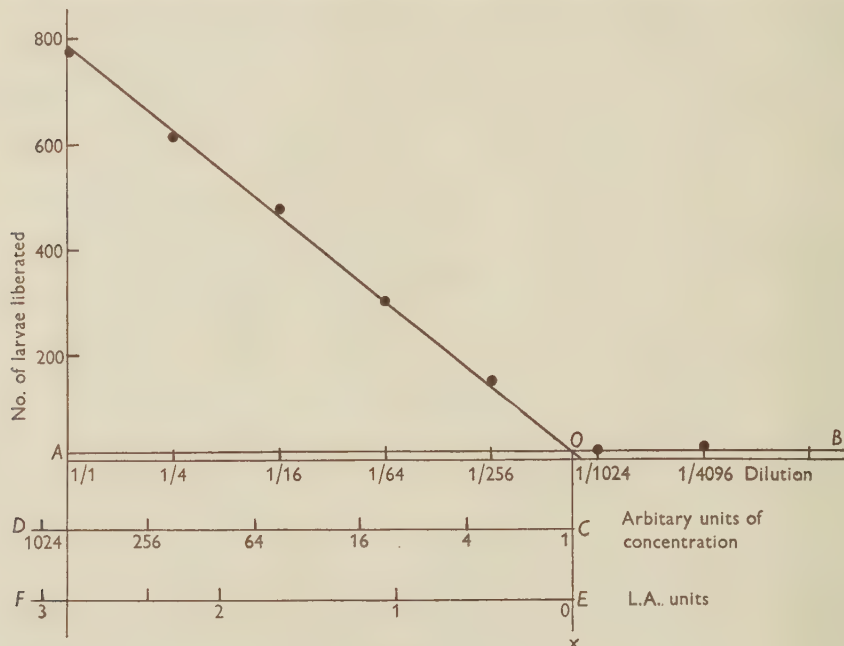


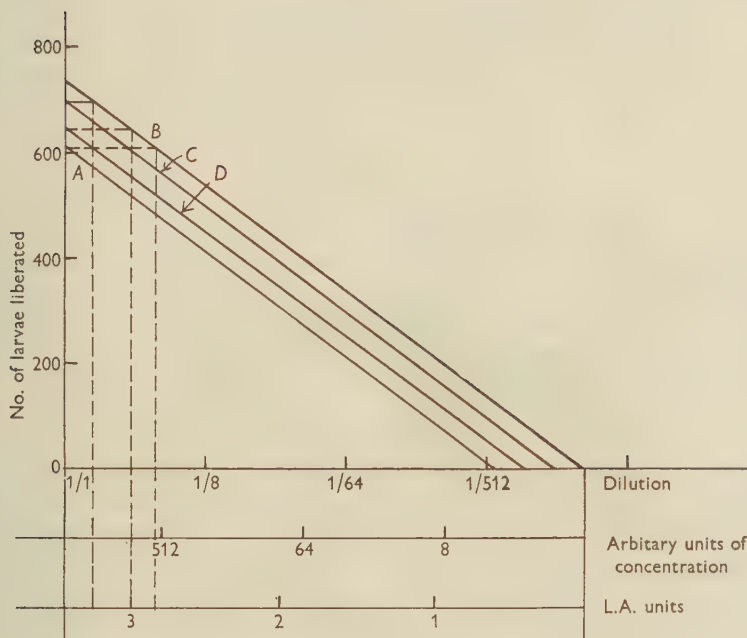
Fig. 4. Derivation of L.A. values.

It will now be seen that the strength of the original undiluted sample is a little less than 1024 arbitrary units. To derive this value more accurately it is convenient to construct a new scale EF on which log values of the scale CD are marked. On this scale it is possible to read L.A. values directly, the L.A. value of the undiluted diffusate in question being 2.85. The antilog of this value (929.4) is its strength in arbitrary units of concentration. Knowing the L.A. value of a single diffusate three alternatives are possible if further samples need standardizing. (1) The known sample can be used as a standard to obtain the L.A. value of any other since hatch is proportional to L.A. value; thus if n_1 larvae emerge in a diffusate of known L.A. value and n_2 from an unknown, then the L.A. of the unknown will be n_2/n_1 that of the standard. (2) Alternatively, a new sample may be standardized without

reference to any previous standard. (3) If a number of samples of diffusate require standardizing, the accuracy of the process can be increased if all the diffusates are put up in similar graded series of dilutions. The dilution curves for each sample can then be examined for parallelism with each other, and provided this is satis-

TABLE 5. *Parameters for dilution curves for samples A-D*

	$\bar{x}$	$\bar{y}$	b	S.E. of b
A	2.5	306	204.9	5.89
B	2.5	439		
C	2.5	402		
D	2.5	354		

Fig. 5. Data of Fig. 3 computed from a pooled estimate of b .

factory a mean regression coefficient can be computed for all lines, the position of each line being governed only by its $\bar{x}$ and $\bar{y}$ value. This procedure has been carried out on the data from the four samples referred to in Fig. 3. The data for each line are set out in Table 5, and the graphs using the new pooled estimate of b are given in Fig. 5. The strongest diffusate B can now be seen to have an L.A. value of 3.42, and expressing A, C and D in terms of it, L.A. values of 2.87, 3.18 and 3.00 respectively are obtained.

In order to compare results using each of these three methods, they were applied to the data for cysts A to D of Fig. 3. (a) The hatches in undiluted samples of

A, *C* and *D* were compared with that of the strongest diffusate *B*, the L.A. value being derived by simple proportion. (*b*) Each sample was itself put up in a geometric series of dilutions and its L.A. value computed absolutely. (*c*) The L.A. values of *A*, *C* and *D* were read off on the L.A. scale of Fig. 5. These results are set out in Table 6. The three columns represent values obtained by the above three methods and are referred to as 1, 2 and 3 respectively. It will be seen that methods 2 and 3 agree better with each other than with 1, which suggests that comparison of a sample of diffusate with a single graded series is probably less precise than an estimate based on either its own dilution curve or a mean value of *b* computed from two or more dilution curves.

TABLE 6. *Comparison of L.A. values derived by three methods*

	Method		
	1	2	3
<i>A</i>	2.77	2.86	2.87
<i>B</i>	3.44	3.44	3.42
<i>C</i>	3.34	3.11	3.18
<i>D</i>	3.12	2.88	3.00

Preparation of diffusates of known L.A. values

Knowing the L.A. value of a diffusate it is possible to modify it by dilution. The difference between the recorded L.A. value and that required is the logarithm of the degree of dilution needed. Thus if the observed L.A. is 2.4 and a value of 2.0 is needed, the difference between the two is 0.4; the antilog of this is 2.51. The diffusate should therefore be diluted to 1/2.51 to give the required value.

LIMITATIONS ON THE BIO-ASSAY METHODS

It will be seen that the method outlined depends on the linear form of the curve obtained when cysts are exposed to a geometric series of dilutions of root diffusate. It is obvious that the linearity of this curve only holds between certain limits. When diffusate is progressively diluted a point is reached at which its action is equal to that of water and beyond which the hatch remains more or less constant whatever the degree of dilution; the curve will consequently flatten out and linearity will be lost. On the other hand, no diffusate can extract from a batch of cysts more larvae than are actually present; hence if the diffusate is sufficiently strong at the start of the experiment the first few observations may depart from linearity. Neither of these limitations is of great practical importance provided that the plotted points are examined with some care. The flattening of the curve near the water line can be readily detected, and values beyond this omitted when fitting a regression line. Lack of linearity at the upper end is less frequent but can usually be detected by visual inspection. It very rarely occurs below L.A. 3.5, although some cysts sometimes show lack of linearity at lower L.A. values. Provided these two limitations on linearity are recognized, the method outlined is capable of yielding consistently valuable information on root diffusate strengths.

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THE INFLUENCE OF THE HOST ON THE DIMENSIONS OF THE PLANT PARASITIC NEMATODE, *DITYLENCHUS DESTRUCTOR*

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(With 2 Text-figures)

Measurement data and body ratios from eleven populations of *Ditylenchus destructor* are presented. The data for lengths of males and females have been analysed for variance, and many differences between population means are shown to be significant. The essential importance of structural characters and the care with which sizes and dimensional ratios must be used, in the identification of a species, is emphasized.

INTRODUCTION

The establishment of a species and its differentiation from others, some of which may be very closely related to it, is dependent on the recognition of a combination of morphological characters unique to the species. The characters will, of course, be subject to the normal variation such as one finds in any population of organisms, but such variation will be of degree only and not of kind.

In the field of free-living nematodes, Ludwig (1938), studying the species *Rhabditis teres* (which is a synonym of *R. terricola* Dujardin, 1845), found, when culturing eelworms on nutrient agar, that alteration of various constituents of the medium caused changes in certain morphological features and dimensional ratios of the organisms. Some changes were slight but others were more striking; for instance, the spicule length was increased considerably when certain plant juices were added to the medium.

Stephenson (1942), working with *R. terrestris*, found similar variations in size and relative dimensions which were correlated with changes in the culture medium.

During experiments made by the writer on the host range of *Ditylenchus destructor* (Goodey, 1951, 1952), it was noticed that there were obvious variations in the size of the eelworms when they were infesting different hosts. This paper demonstrates the range of these variations and shows that the host exerts a considerable effect on the body lengths of the parasite.

MATERIAL AND METHODS

Specimens of *D. destructor* were collected from plants which had been used in the author's host transfer experiments (Goodey, 1951, 1952). They were relaxed by heating at 70° C. and quickly transferred to fixative, F.A. 4 : 10 (Franklin & Goodey,

1949). The eelworms were stored in this fixative, which causes no more than 1 % shrinkage, until they were measured. Specimens were mounted in fixative on slides whose covers were supported on glass rod thick enough to prevent any pressure on the nematodes. The covers were sealed temporarily by painting with the wick of a recently extinguished candle. Camera lucida drawings were made of the outlines of the eelworms, and the positions of the oesophago-intestinal junction, the vulva, anus and extent of the gonads were marked. Dimensions were obtained by measuring the drawings with lead fuse-wire bent to the shape of the eelworms.

Random samples of twenty-five males and twenty-five females from each population were measured except where these numbers were fewer, when the whole adult population was measured. Measurements were made of eelworms in populations from eleven different sources, all carefully examined under oil immersion to confirm that each was *D. destructor*.

RESULTS

The lengths and the usual body ratios are set out in Tables 1, 2 and 3. In Tables 1 and 2 the data are arranged to show the means and the ranges obtaining within each population, while in Table 3 the means obtained from all the writer's data are compared with means derived from Thorne's (1945) measurements.

A consideration of these data shows that, in general, females vary in length more than males, that the female is relatively stout, though she becomes slimmer in some hosts and that the size of the gonads of both sexes is influenced by the host. The relative position of the vulva, which is a structural feature, is, however, practically unaffected.

The data for lengths have been subjected to analysis of variance, separately for males and females. The whole of the measurement data presented could have been analysed item by item. But since the object of these observations was not primarily statistical but biological, it was not considered that the arguments used in this paper would be materially affected by the use of additional statistical backing, however interesting in themselves the results might have been.

The analyses show that the effect of the host on the length of both male and female eelworms is highly significant at the 1 % level. The mean lengths, with significant differences at the 5 % level, are set out in Table 4. There is less overall variation in length in males than in females, the greatest difference amounting to 45 % of the mean for males compared with 64 % for females.

The times that the different populations lived on their particular hosts varied. The measurement data were by-products of experiments designed for other purposes. With this qualification one can consider the results obtained, using the data from the eelworms infesting potato as a standard with which to compare the others.

Females. Eelworms from iris are significantly longer than those from potato; when transferred from potato to iris the length increases but not significantly so, though when eelworms are transferred from iris to potato they become shorter

TABLE 1. *Dimensions of twelve populations of female Ditylenchus destructor showing means and ranges*

Population	<i>n</i>	<i>l</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>V</i>
1. Transferred from potato→potato	25	995 (830-1270)	27.5 (18-38)	7.0 (5-10)	15.4 (12-20)	61.4 (38-76) (73-90) 8.7 (7-12)
2. Transferred from iris→iris	25	1139 (920-1340)	29.5 (24-37)	7.7 (4-10)	16.0 (11-22)	61.5 (40-78) (76-82) 9.0 (6-13)
3. Transferred from potato→iris	25	1037 (900-1260)	32.4 (27-39)	6.7 (6-8)	16.1 (13-23)	45.7 (28-64) (73-88) 8.3 (7-10)
4. Transferred from iris→potato	25	1073 (880-1380)	29.9 (24-37)	7.3 (6-9)	18.4 (15-25)	57.5 (39-74) (73-83) 8.6 (6-14)
5. Transferred from potato→mint	25	975 (810-1180)	34.4 (27-49)	6.6 (6-8)	15.2 (11-20)	49.9 (39-70) (77-82) 8.1 (7-12)
6. Transferred from potato→mint→potato	25	1084 (960-1190)	24.1 (21-27)	7.7 (6-10)	19.9 (15-25)	67.3 (60-76) (80-84) 9.5 (8-11)
7. Transferred from iris→mint	14	926 (850-1050)	37.2 (29-46)	6.2 (5-7)	14.2 (11-22)	43.3 (34-51) (73-82) 8.6 (7-12)
8. Transferred from potato→ <i>Stachys palustris</i>	25	1307 (1010-1500)	31.4 (23-42)	8.0 (6-11)	16.5 (12-22)	54.4 (37-74) (78-84) 7.6 (6-10)
9. Transferred from hop→iris	12	829 (690-970)	37.6 (33-45)	6.6 (5-10)	16.2 (12-24)	38.8 (28-59) (78-87) 8.0 (6-10)
10. Transferred from iris→mint→hop	11	895 (770-1010)	33.5 (28-38)	6.0 (5-7)	14.2 (9-19)	51.1 (42-63) (77-82) 7.1 (4-11)
11. Transferred from potato→ <i>Tropaeolum polyphyllum</i>	25	1513 (1190-1890)	29.8 (25-38)	9.2 (7-12)	19.5 (15-30)	58.2 (51-66) (79-85) 8.0 (6-9)
Thorne's	?	1100 (800-1400)	32.5 (30-35)	9.0 (8-10)	17.5 (15-20)	65 (78-83) 10

TABLE 2. *Dimensions of twelve populations of male Ditylenchus destructor showing means and ranges*

Population	<i>n</i>	<i>l</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>T</i>
1. Transferred from potato→potato	25	1025 (790-1250)	32.4 (25-41)	6.5 (5-8)	14.4 (13-17)	66.7 (56-75)
2. Transferred from iris→iris	25	944 (780-1040)	31.1 (25-37)	7.5 (6-9)	13.4 (11-17)	65.1 (42-80)
3. Transferred from potato→iris	25	954 (850-1090)	39.1 (32-50)	6.6 (6-8)	14.2 (12-17)	59.6 (46-77)
4. Transferred from iris→potato	25	972 (810-1220)	32.9 (29-44)	6.8 (6-8)	14.7 (13-18)	66.0 (53-76)
5. Transferred from potato→mint	25	934 (810-1080)	37.2 (31-44)	6.5 (6-7)	13.0 (11-15)	57.3 (43-68)
6. Transferred from potato→mint→potato	25	988 (850-1140)	30.7 (24-38)	7.5 (7-8)	14.8 (13-18)	72.9 (65-84)
7. Transferred from iris→mint	12	868 (760-990)	37.4 (30-44)	6.4 (5-7)	12.9 (11-15)	59.5 (54-65)
8. Transferred from potato→ <i>Stachys palustris</i>	23	990 (790-1160)	36.4 (31-44)	6.6 (5-7)	14.7 (13-21)	56.8 (40-73)
9. Transferred from hop→iris	12	783 (670-910)	39.7 (31-46)	6.2 (5-8)	12.8 (11-15)	49.5 (40-60)
10. Transferred from iris→mint→hop	9	837 (760-1020)	40.3 (32-49)	5.5 (4-6)	13.6 (12-15)	58.1 (53-64)
11. Transferred from potato→ <i>Tropaeolum polyphyllum</i>	25	1216 (950-1350)	38.4 (28-47)	7.3 (6-11)	15.4 (12-19)	67.4 (51-76)
Thorne's	?	1050 (800-1300)	37.5 (34-40)	7.5 (7-8)	14.0 (12-16)	76.4 (73-80)

TABLE 3. *Summarized data from author's populations compared with those from Thorne's*

Population	Sex	<i>n</i>	<i>l</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>V</i>
Author's	♀	237	1070	31.6	7.2	16.5	⁵⁴ 80.3 ⁸
Thorne's	♀	?	1100	32.5	9.0	17.5	⁶⁵ 80.3 ¹⁰
							<i>T</i>
Author's	♂	231	960	35.1	6.7	14.0	65.1
Thorne's	♂	?	1050	37.0	7.5	14.0	76.4

Notes for tables. *l*=length in μ ; *a*=length÷greatest width; *b*=length÷oesophageal length; *c*=length÷tail length; *V*=position of vulva as percentage of body length, the prefix and suffix indicating the anterior and posterior extents of the ovary; *T*=extent of testis as a percentage of body length; *n*=number of individuals contributing to the mean.

by an amount which is just significant. Transfer from potato to mint makes little difference to length, but from iris to mint the change is greater and highly significant. Transfer from potato to *Stachys palustris* causes a considerable increase, from 995 to 1307 μ , and to *Tropaeolum polyphyllum* the increase in length is greater still, up to 1513 μ .

Males. Those of the iris population show a trend diametrically different from the females, being significantly shorter than those of the potato population. Transfer from potato to iris causes a significant shortening, but from iris to potato the lengthening is not significant.

TABLE 4. *Mean lengths with standard deviations and significant differences applicable to differences between the lengths*

Populations	Females			Males		
	<i>n</i>	Mean length (μ)	Standard deviation (μ)	<i>n</i>	Mean length (μ)	Standard deviation (μ)
1. Transferred from potato→potato	25	995	132	25	1025	111
2. Transferred from iris→iris	25	1139	123	25	944	83
3. Transferred from potato→iris	25	1037	94	25	954	65
4. Transferred from iris→potato	25	1073	113	25	972	116
5. Transferred from potato→mint	25	975	100	25	934	72
6. Transferred from potato→mint→potato	25	1084	65	25	988	66
7. Transferred from iris→mint	14	926	56	12	868	63
8. Transferred from potato→ <i>Stachys palustris</i>	25	1307	137	23	991	115
9. Transferred from hop→iris	12	829	97	12	783	80
10. Transferred from iris→mint→hop	11	895	87	9	837	79
11. Transferred from potato→ <i>Tropaeolum polyphyllum</i>	25	1513	156	25	1216	117
	<i>n</i> ₁	<i>n</i> ₂	S.D. (μ)	<i>n</i> ₁	<i>n</i> ₂	S.D. (μ)
Significant differences ($P=0.05$) applicable to differences between length means based on <i>n</i> ₁ and <i>n</i> ₂	25	25	65	25	25	51
	25	14	75	25	23	53
	25	12	79	25	12	64
	25	11	81	25	9	71
	14	12	88	23	12	65
	14	11	90	23	9	71
	12	11	93	12	12	74
				12	9	80

In addition the data for each population have been set out diagrammatically in Figs. 1 and 2. No attempt has been made to indicate the tapering shape of the eelworms, but each unit represents a 'mean eelworm'.

The units have been arranged in ascending order of lengths and are numbered with the same population numbers used in the tables. Since the position of the vulva, calculated as a percentage of body length, was remarkably constant, this was used as a base line for the females, but for the males the tail tip has been used as the base. The order of the populations in the two series is not the same.

Females. In general the longer the eelworm the fatter it is; this is borne out in the relative constancy of the values of *a* for the females. There are exceptions to

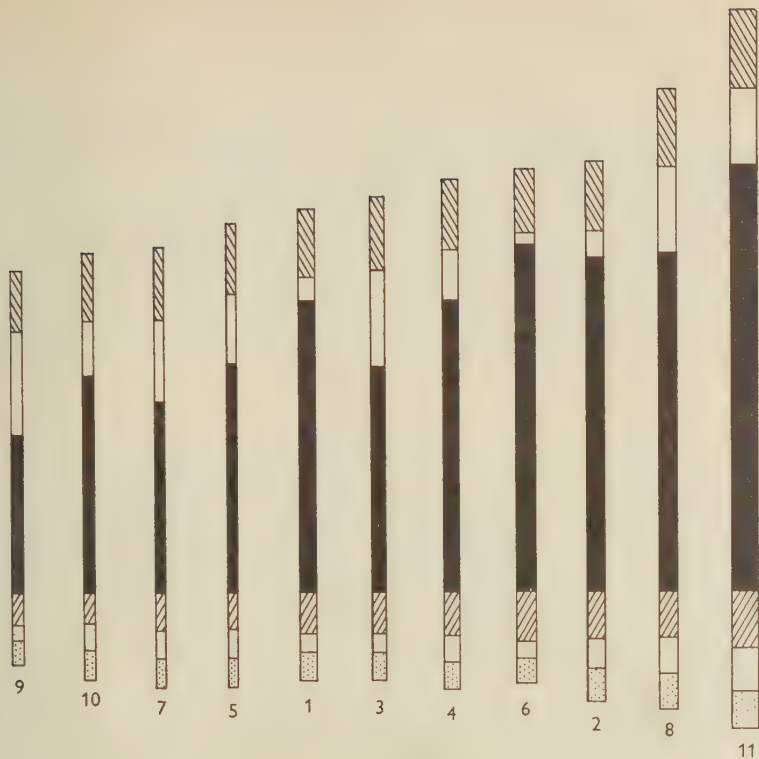


Fig. 1. Diagram of female 'mean eelworms'; populations numbered as in Tables 1 and 2.



Fig. 2. Diagram of male 'mean eelworms'; populations numbered as in Tables 1 and 2.

▨ = oesophagus; ■ = gonad; ▨ = post-vulval sac; ▤ = tail.

the tendency of increase of body diameter with length, as shown by populations 1 and 6, where, for instance, the eelworms of these populations have larger gonads. Other features are the relative constancy of the lengths of the oesophagus, the post-vulval sac and the tail.

Males. Similar tendencies are shown by the males with similar exceptions. Populations 2 and 6 are fatter and have correspondingly greater gonads. The oesophageal lengths and tail lengths are relatively constant.

In making these observations it must be emphasized that they are the indications that can be derived from the figures; no wider significance is claimed for them.

DISCUSSION

The evidence from transfer experiments and the critical microscopical examination of eelworms from the various hosts showed that all were *Ditylenchus destructor*.

In spite of the picture of considerable diversity manifested in the lengths and sizes of gonads, the means of all the data give figures which compare remarkably closely with those given by Thorne when he established the species, *D. destructor* (Table 3). The fact that one can demonstrate statistical differences between populations of *D. destructor* infesting different hosts adds weight to the patency of this diversity.

A species is essentially a dynamic group of individuals, and the observations recorded in this paper emphasize the point. Thus in the identification of eelworms, particularly the plant parasitic species, although the important features are the anatomical and structural ones, factors such as size, dimensional ratios and the extent of the gonads must be treated with care. It follows, too, that the systematic nematologist should adopt what is essentially an ecological attitude, dealing not merely with dead nematodes but treating them as living organisms, studying their behaviour and the reaction of their hosts as well as their appearance.

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STUDIES IN THE RELATIONSHIP OF EELWORMS AND BACTERIA TO CERTAIN PLANT DISEASES

I. THE ETIOLOGY OF STRAWBERRY CAULIFLOWER DISEASE

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(With Plate 17)

Two types of organism are known to be present in strawberry plants suffering from cauliflower disease, namely, eelworms, *Aphelenchoides ritzema-bosi* and/or *Aphelenchoides fragariae*, and a bacterium, *Corynebacterium fascians*. Neither type, inoculated separately, has consistently reproduced the disease.

An experimental investigation of a possible obligate etiological relationship between the eelworm and the bacterium is described, in which *Aphelenchoides ritzema-bosi* and several strains of *Corynebacterium fascians* were inoculated together and separately.

No abnormalities appeared in the absence of the eelworm. Typical cauliflower symptoms were reproduced only by a combination of the eelworm and strains of *C. fascians* originally isolated from cauliflower strawberries. Small alamine leaves and enations appeared in all treatments where the eelworm was introduced, including those where no bacteria were co-inoculated. Subsequent isolations, however, demonstrated the presence of contaminant strains of *C. fascians* in the latter plants. The severity of the enation symptom was related to the presence of a particular strain of *C. fascians*, but the alamine leaves showed no such correlation. Evidence on the source of the contaminant strains found in the experimental plants is discussed.

In parallel field investigations it was established that eelworm-infested strawberries showing no cauliflower symptom frequently bore alamine leaves, with which *C. fascians* was consistently associated. It is suggested therefore that cauliflower is simply the least frequent of a range of symptoms of a bacterial disease endemic in strawberry plants infested with *Aphelenchoides* spp.

INTRODUCTION

The term cauliflower disease was proposed by Ritzema-Bos (1889) to describe a malformation of the inflorescence and shortening and thickening of the aerial parts of strawberry plants sent from Kent by Miss E. A. Ormerod. He considered an ectoparasitic nematode which he consistently found in the diseased plants to be the causal agent, and later (Ritzema-Bos, 1891) he described the species and named it *Aphelenchus fragariae* n.sp. Since then the disease has occurred sporadically in England and on the continent of Europe and has been reported from North America (Christie & Stevens, 1933) as an occasional variant from spring dwarf, a strawberry disease attributed to the same nematode.

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In the most severe examples of cauliflower disease, one or more of the crowns are reduced to stunted fleshy rosettes about 1 cm. in height (Pl. 17, Fig. 1). The bulk of such a structure is derived from leaf tissue, mainly in the form of short swollen petioles, although undifferentiated gall tissue and rudimentary blossoms may also be present. Many intermediate forms exist between this and the healthy plant. In the present paper the term 'cauliflower' is restricted to the more extreme symptoms and 'near-cauliflower' used to indicate the less severe forms where the leaves, although clearly recognizable as such, have short fat petioles and small puckered laminae. Small outgrowths of gall tissue may occur on the leaves and flower trusses of these plants also.

A. fragariae has also been reported as an ectoparasite on strawberries suffering from red plant disease. This condition, so called because of the marked red coloration present on certain varieties, notably Royal Sovereign, is characterized by reduced leaf laminae borne on slender, tapering, hairless petioles and by rudimentary blossom trusses. These symptoms are more severe towards the centre of the crown, although the growing point itself usually remains alive.

Considerable observational and experimental work on the cauliflower and red plant diseases was carried out at Long Ashton in the years 1922-9. Ballard & Peren (1924) gave full descriptions of both and drew attention to many points of similarity between them. In a series of etiological investigations Staniland and co-workers (1925-9) were unable to reproduce either cauliflower or red plant by inoculation of eelworms, and concluded that there was no evidence that *A. fragariae* was the sole cause of these diseases. This conclusion was supported by the discovery of moderate populations of eelworms in many apparently healthy plants, including some used as controls in the experiments.

Dicker (1948), using a species now known to be *A. ritzema-bosi*, was also unable to reproduce cauliflower symptoms. Nearly all his inoculated plants developed some leaves distorted by eelworm feeding, the rough brown patches thus induced on the upper surface of the laminae now being known as 'feeding areas'. Such damage was typically followed by death of the primary crown, which in turn stimulated the production of a number of weak secondary crowns, giving barren, open-centred plants. Many of these plants produced small leaves with thin, red, glabrous petioles on the secondary crowns and thus resembled red plants in many respects. There were differences, however, between such plants and those regarded as typical by the Long Ashton workers, notably the absence of a progressive attenuation of the leaves and crown.

Lacey (1936, 1942) threw new light on the etiology of the cauliflower and red plant diseases by the isolation of a Gram-positive bacterium which she had also found associated with fasciated sweet peas and similar hyperplastic diseases of other plants. The same organism, now known as *Corynebacterium fascians* (Tilford) Dowson, was reported independently by Tilford (1936) in America as causing fasciation of sweet pea and leafy gall of chrysanthemum. Lacey (1936, 1939) readily induced leafy galls and cauliflower-like tissues in a wide range of host plants with

C. fascians, either by direct inoculation or by watering it on the soil. Such tests on strawberry, however, were not uniformly successful, for although symptoms resembling those of red plant or cauliflower disease could occasionally be produced on seedling plants, inoculation of runner plants gave inconclusive results (Lacey, 1942).

It is apparent, therefore, that eelworms have been associated with three symptom-types on strawberry, viz. cauliflower, red plant and 'open-centre' plants, of which only the last has been reproduced by inoculation. *C. fascians* has also been associated with cauliflower and red plant symptoms but has not been shown conclusively to be the cause of either.

As neither of these lines of approach satisfactorily explained the etiology of cauliflower disease, the possibility of an obligate etiological association between the eelworm and the bacterium was considered, and formed the subject of the investigation described below.

SYNONYMY AND DISTRIBUTION OF EELWORMS

Franklin (1950) showed that although only *A. fragariae* had been recorded in the literature, there were in fact two species of this genus (now known as *Aphelenchoides*), which commonly attacked strawberry plants, namely, *A. fragariae* (Ritzema-Bos, 1891) Christie, 1932, syn. *A. olesistus* (Ritzema-Bos, 1893) Steiner, 1932 and *A. ritzema-bosi* (Schwartz, 1911) Steiner, 1932. After examining strawberries from many localities she concluded that both species were widespread in the British Isles, sometimes occurring together in the same plant, and suggested that their identity had been confused in the past. Most of the material examined in the present investigation came from south-east England; at first only *A. ritzema-bosi* was found in this area, but *A. fragariae* has recently been introduced in plants from outside sources.

OBSERVATIONS ON THE ASSOCIATION OF *APHELENCHOIDES* SPP. AND

CORYNEBACTERIUM FASCIANS WITH CAULIFLOWER AND RED PLANT DISEASES

Field observations in 1949-50 showed that cauliflower disease was rare in south-east England, except in the variety Mme Lefebvre, and that red plant disease was virtually absent. Open-centre plants were commonly found, however, and this was therefore regarded as the more typical form of eelworm injury in the area. This conclusion was supported by further eelworm inoculation experiments on several strawberry varieties, when the open-centre plant was again the only symptom-type observed.

In the present investigation both species of eelworm have been found associated with cauliflower, but so far only *Aphelenchoides fragariae* with red plant disease. Smaller populations were encountered in cauliflower strawberries than in plants showing feeding areas only. The ectoparasitic habit of the eelworm was confirmed by staining portions of cauliflower crowns in acid fuchsin/lactophenol and clearing in phenol.

Isolations of *Corynebacterium fascians* were attempted from several red plant and cauliflower strawberries. Isolation was facilitated by the use of a nutrient agar (Difco) containing 5 % sucrose and 0.02 % potassium tellurate. Before pouring the medium, sufficient potassium dichromate solution (sterilized separately) was added to give a final concentration of 0.0033 %. Isolates were identified by colony characteristics, Gram stain and pathogenicity to sweet peas (Lacey, 1936).

Thirteen strawberries with cauliflower or near-cauliflower symptoms were examined and, in each, *C. fascians* was found in large numbers. These plants were from five localities, and included the varieties Mme Lefebvre, Paxton, Climax and Royal Sovereign. The only Royal Sovereign plant examined came from Cornwall in 1949 as a typical red plant and developed near-cauliflower symptoms the following year. Four red plants were examined, three Climax and one Royal Sovereign, and *C. fascians* was readily isolated from each. Seven cauliflower strains of the bacterium were tested against sweet peas and found to be fairly pathogenic (Pl. 17, fig. 2); the strains from red plants proved much less so.

INOCULATION EXPERIMENTS

In 1950-1 two experiments were begun, in autumn and spring respectively, to test the effect of eelworms and bacteria, together and separately, compared with uninoculated controls. Eelworm-free plants of the variety Mme Lefebvre (Malling S92) were raised in 1950 in unheated insectaries. In an attempt to exclude soil from the crowns the steam-sterilized potting compost was covered with half an inch of autoclaved sand, and water was carefully applied through a tube by gravity feed from a reservoir.

Eelworms (*Aphelenchoides ritzema-bosi*) were obtained from 'wool' on folded leaflets of potted Royal Sovereign runner plants, originating from parents inoculated in the field in 1949-50. The eelworm inoculum was immersed in a bactericide before use. Mercuric chloride at 0.05 % for 30 min. (Christie & Crossman, 1936) was found to be highly nematocidal, but immersion for 72 hr. in an aqueous solution at 3.75 p.p.m. of ethoxy ethyl mercury chloride gave a satisfactory degree of bactericidal action and killed fewer than 10 % of the eelworms. Details of this and other sterilization techniques will be published elsewhere. After immersion the eelworms were washed four times in sterile water, kept overnight and washed twice more.

The sources and strains of *Corynebacterium fascians* used in the experiments were as follows:

- (1) Strains C1 and C6. Isolated from cauliflower tissues of Mme Lefebvre strawberries, June 1950.
- (2) Strain P30. From Dr M. S. Lacey, May 1950; originally from sweet pea.

All three strains were pathogenic to sweet pea, particularly P30 (Pl. 17, fig. 2).

METHODS OF INOCULATION

All inocula were applied as aqueous suspensions. Approximately 125 eelworms were given in all treatments except 3*b*, 3*c* and 3*d* in Exp. 1, where some loss occurred in the mixing process. Bacterial suspensions were prepared from 7–10 days' growth on nutrient agar + 2 % glycerine.

Experiment 1. Inoculated 9–14 December 1950, five replicates of each treatment.

Treatment 1: C6 sprayed on crown.

2: eelworms dropped on crown.

3*a*: treatment 2, followed in 5 days by treatment 1.

3*b*: mixed suspension of eelworms and C6.

3*c*: mixed suspension of eelworms and C1.

3*d*: mixed suspension of eelworms and P30.

4: no treatment.

Experiment 2. Inoculated 22–23 March and reinoculated 11–12 April. Ten replicates of each treatment.

Treatment 1: C6 dropped on crown.

2: eelworms dropped on crown.

3: treatment 1, followed in 24 hr. by treatment 2.

4: sterile water dropped on crown.

Symptoms recorded

The experimental plants were recorded from mid-May to late September. Symptoms were classified in the following five groups, which are listed in approximate order of appearance.

(*a*) *Feeding areas.* Superficial injury to the upper surface of the young leaf, caused by the ectoparasitic feeding of many eelworms (Pl. 17, fig. 3).

(*b*) *Enations.* Well-marked enations are often present on field plants with near-cauliflower symptoms, but most of those on the experimental plants were less severe, often amounting to little more than a roughening of the epidermis. They first appeared on petioles and peduncles of primary crowns (Pl. 17, fig. 4) and later along the veins on the lower surface of leaves, especially those bearing feeding areas. Leaf vein enations occasionally developed small amounts of gall tissue.

(*c*) *Alaminate leaves.* Typically a rosette of very small petioles, each $\frac{1}{2}$ –1 cm. long, more or less glabrous, often browned superficially and tapered to a point, which represented an extremely reduced lamina (Pl. 17, fig. 5). Identical structures occasionally form part of a cauliflower crown (Ballard & Peren, 1924), but those recorded in the experiments were immediately preceded by leaves of normal size, although often bearing a feeding area and/or enations. Here, therefore, they were symptomatic of a sharp check in the normal cycle of leaf production. Typical alamate leaves died in 2–4 weeks without undergoing any change in structure.

The growing point itself either died too, produced a cauliflower crown, or gradually resumed normal growth.

(d) *Secondary crowns*. A stimulus to secondary crown production is in itself a symptom; the total number produced, normal and abnormal, was therefore recorded. Alamine-leaved plants whose primary growing point died usually developed 3-4 secondary crowns, thus becoming typical open-centre plants.

(e) *Cauliflowers*. Typical cauliflower and near-cauliflower crowns are grouped in this category. In Exp. 2 the alamine leaves of two crowns produced small amounts

TABLE 1. *Symptoms recorded on strawberry plants after inoculation with Aphelenchoides ritzema-bosi and/or Corynebacterium fascians*

Treatment	No. of plants bearing							
	Alaminate leaves	Enations	Cauliflowers			No. of crowns bearing		
			primary and/or secondary crowns	In secondary crowns only	No. of secondary crowns per treatment	Alaminate leaves	Enations	Cauliflower
<i>Exp. 1 (5 plants per treatment):</i>								
1. C6	0	0	0	0	2	0	0	0
2. Eelworm	4	3	0	0	15	10	3	0
3 <i>a.</i> Eelworm followed by C6	5	5	2	1	23	10	15	5
3 <i>b.</i> Eelworm plus C6	5	5	3	2	26	13	17	6
3 <i>c.</i> Eelworm plus C1	3	3	2	2	15	4	3	4
3 <i>d.</i> Eelworm plus P30	4	3	0	0	16	9	6	0
4. Control	0	0	0	0	3	0	0	0
<i>Exp. 2 (10 plants per treatment):</i>								
1. C6	0	0	0	0	3	0	0	0
2. Eelworm	8	3	2*	0	30	14	3	2*
3. C6 followed by eelworm	9	6	6	5	40	20	12	11
4. Control	0	0	0	0	4	0	0	0

* Small and atypical.

of gall tissue before dying. These were considered to differ from the cauliflower symptom only in degree and were therefore included in this category; they are marked with an asterisk in Table 1. Cauliflower crowns arose in two ways, either following alamine leaves or growing direct from a secondary crown bud. Whatever their origin they were often temporary structures, either dying after a few weeks or, more rarely, gradually resuming more or less normal growth.

Records of the above symptoms, taken at weekly to fortnightly intervals, are summarized in Table 1. Separate records were made of primary and secondary crowns on each plant. The severity of symptoms is most clearly shown by the number of crowns affected (columns 6-8 inclusive). For all treatments the number of secondary crowns was submitted to an analysis of variance. For all other

symptoms the significance of the results of treatments 2-3 only was determined by means of an exact test, the probability calculated being that of a result as extreme as that observed, or more so. In one instance the χ^2 test was used instead. In Exp. 1 symptoms first appeared in mid-May, 3-4 weeks earlier than in Exp. 2, otherwise there was no essential difference between them. The results of both experiments are therefore discussed together.

The bacterium caused no abnormality when inoculated without eelworms, the plants remaining indistinguishable from the controls. The same negative result was obtained when it was prick-inoculated into additional plants not included in the experiments. Whenever the eelworm was inoculated there was a highly significant stimulus to the production of secondary crowns ($P < 0.001$). The incidence of alamate leaves could be correlated only with the presence of the eelworm, and at this stage of the investigation appeared to occur independently of the bacterium. Enations also occurred in all treatments including eelworm, but their incidence was significantly increased where strain C6 of the bacterium was present ($\chi^2 = 20.55$, $P < 0.001$, Exp. 1; $P = 0.035$, Exp. 2).

Typical cauliflower crowns developed only in plants inoculated with eelworms and the cauliflower strains of *C. fascians*, C6 and C1. In Exp. 1 the occurrence of seven cauliflowers in the fifteen plants inoculated with these two strains was significant when compared with their absence in all other eelworm-inoculated plants ($P = 0.013$). In Exp. 2 a significantly higher number of cauliflower crowns resulted when strain C6 was co-inoculated with the eelworm (treatment 3) than when the eelworm was inoculated alone (treatment 2), even when the cauliflowers in the latter treatment were regarded as typical ($P = 0.022$). On the basis of number of plants affected this difference was also significant for secondary crown cauliflowers ($P = 0.016$), but not for total cauliflower crowns ($P = 0.085$).

These results show clearly that while the eelworm was essential to the production of all symptoms, typical cauliflower symptoms occurred only when certain strains of *C. fascians* (i.e. those isolated from cauliflower plants) were associated with it. It will be noted that no cauliflower crowns were formed in plants inoculated with the sweet-pea strain P30, although this was very pathogenic to sweet pea. The highly significant increase in enations occurring when strain C6 was inoculated indicated that the bacterium played an essential role in the production of this symptom also. It will be apparent later that this conclusion is not invalidated by the occasional occurrence of enations in plants inoculated with eelworm alone. The fact that no comparable increase in enations occurred when the other cauliflower strain (C1) was inoculated indicated that differences between strains of *C. fascians* are complex and not solely related to the production of cauliflower symptoms.

It was possible to conclude from the more or less uniform distribution of alamate leaves in all the eelworm treatments, including those in which no bacteria were inoculated, that this symptom was caused entirely by the eelworm. For the

following reasons, however, it was suspected that the plants inoculated with eelworm alone were contaminated with *C. fascians*.

(1) The alamate leaves did not seem to differ fundamentally from the basic cauliflower symptom, and it was considered unlikely that they could result entirely from the ectoparasitic feeding of an eelworm. In addition, it was noted that Lacey (1948) had already found *C. fascians* associated with similar structures in dwarfed plants of *Viola* sp. and *Primula* sp.

(2) The occurrence of occasional enations and atypical cauliflower symptoms in the plants inoculated with eelworm alone could not be reconciled with the other experimental evidence associating these symptoms with the bacterium.

Test isolations were therefore made from alamate leaves of plants of treatments 2 and 3. *Corynebacterium fascians* was found in large numbers in both cases and several isolates from treatment 2 were positively identified by their slight but distinct pathogenicity to sweet peas. The presence of contaminant strains in treatment 2 provided an explanation of the anomalous results following inoculation with eelworm alone, and in so doing strengthened the conclusions already drawn concerning the essential role of *C. fascians* in the etiology of the enation and cauliflower symptoms. They further suggested that the alamate leaf might also result from the joint activity of the eelworm and bacterium. This view is supported by observations and isolations described below, made on field strawberries; final proof of this, however, must wait until the effect of eelworms on sterile plants is known.

FURTHER FIELD OBSERVATIONS

Before the death of the crown and the formation of open-centre plants, many of the experimental strawberries without cauliflower symptoms could be distinguished from the controls only by the presence of feeding areas, enations and alamate leaves. Plants with all these symptoms but otherwise presenting a superficially normal appearance had not previously been noted in eelworm-infested field strawberries, nor, apparently, had they been described in the literature. It was thought possible, therefore, that they represented an artificial response to experimental conditions.

Parallel field examinations of eelworm-infested strawberries showed, however, that this was not the case. In one typical field a few of the infested plants showed cauliflower symptoms, but the majority appeared comparatively normal apart from reduced vigour and feeding areas. In a high proportion of these plants alamate leaves were readily found and, in a few cases, enations. Later the alamate leaves died, followed usually by the death of the crown, giving open-centre plants. In a few plants the alamate leaves persisted and developed gall tissues similar to those observed in the two eelworm-inoculated plants of Exp. 2. It was thus apparent that the types and succession of symptoms present in the field were identical with those recorded in the experiments.

As in the experimental plants, *C. fascians* was consistently isolated from alamate

leaves of field strawberries showing no other symptom but feeding areas. Two of these strains showed marked pathogenicity to sweet peas (Pl. 17, fig. 6), but most of them were not very active. It was clear, therefore, that this organism was widespread in eelworm-infested strawberries, and not confined to plants with cauliflower symptoms.

THE SOURCE OF CONTAMINANT STRAINS OF *C. fascians*

The three most likely sources of the contaminant strains isolated from the experimental plants are discussed in turn.

(1) *The soil*

Saprophytic *Corynebacterium* spp. are known to occur in the soil (Jensen, 1934). Jacobs & Mohanty (1951), discussing *C. fascians*, suggested that while factors limit its ability to infect '... the pathogen is of common occurrence in the soil'.

The composts used for the experimental strawberries were prepared from steam-sterilized soil. This, apparently, does not guarantee freedom from *C. fascians*, for Lacey (1939) quotes a case where the incidence of leafy gall in *Nicotiana* spp. was frequently correlated with the use of soils in which leafy-gall chrysanthemums had been grown, although the soil had been sterilized by baking.

Whatever the immediate source of these contaminant strains, therefore, it is probable that they came originally from the soil.

(2) *The eelworm inocula*

Eelworms freshly isolated from plants are heavily contaminated with bacteria, and the danger of introducing *Corynebacterium fascians* in this way is very real. In sterility tests carried out on whole and crushed eelworms treated by the pre-inoculation methods, however, *C. fascians* was never observed on the isolation plates. In most cases almost complete sterility was achieved with samples of 200–300 eelworms, while comparable unsterilized controls yielded an estimated minimum of 150,000 bacterial colonies. It is clear, therefore, that *C. fascians* was not introduced with the eelworms.

(3) *The experimental plants*

Five healthy strawberry plants, a surplus from the batch used in the experiments, and two runners from these, were examined for the presence of *C. fascians*. All these plants had been struck into steam-sterilized compost through a layer of autoclaved sand. Test isolations were made from the above-ground portions of the stems and from the dead tissues normally found on the stipules enclosing the terminal buds of strawberry plants.

Colonies of a *Corynebacterium* sp. indistinguishable from *C. fascians* appeared on all isolation plates. In pathogenicity tests none of these isolates caused fasciation of sweet-pea seedlings, although certain responses, e.g. abnormal production of

adventitious roots (Jacobs & Mohanty, 1951), were suggestive. By all other tests the isolates were indistinguishable from virulent strains of *C. fascians*.

Until the identity of the *Corynebacterium* sp. present in the healthy plants has been established it is not possible to decide with certainty the source of the contaminant strains encountered in the experiments. But the above evidence suggests that, despite precautions taken to exclude them, they were present in the experimental plants prior to inoculation.

DISCUSSION

It is evident from past investigations and from the observations and experiments described above, that cauliflower is merely the least frequent of a range of symptoms associated with eelworm attack on strawberries. The experimental evidence shows that two of these symptoms, viz. cauliflower and enations, are not caused by the eelworm alone, but require the association of certain strains of *C. fascians*. There is still insufficient evidence to define the relative roles of the eelworm and the bacterium in the production of these two symptoms, but by analogy with hyperplastic diseases of other hosts, which can be readily reproduced by inoculation with *C. fascians* alone, it is likely that the bacterium is mainly responsible for the cauliflower symptom. The eelworm probably functions as a vector, transporting the bacterium to the meristematic regions of the crown, which, because of their tight enclosure within stipules, are normally inaccessible to it. It is also possible that the eelworms' ectoparasitic mode of feeding may in some way favour the activity of the bacterium.

The experiments provide no conclusive evidence on the etiology of the other symptoms associated with eelworm infestation, e.g. alamate leaves, and the open-centre plants which frequently follow the death of these structures. The widespread distribution of *C. fascians* and its consistent association with the alamate leaf, suggest, however, that this symptom also results from an association of eelworm and bacterium. It is conceivable that the range of symptoms present in eelworm-infested plants is largely a result of variations in *C. fascians*, cauliflower being caused only by the less common but more virulent strains. Evidence of such strain variation is available from the strawberry inoculations and from routine identification tests, when the cauliflower strains were usually found to be more pathogenic to sweet-pea seedlings than those isolated from alamate leaves. Similar variation in the bacterial pathogen may account for the differences between cauliflower and red plant diseases, although here the other eelworm species, *Aphelenchoides fragariae*, and the differing varietal reactions of the host, have also to be considered.

There is evidence suggesting that a similar, though not necessarily obligate association of *Aphelenchoides* spp. and *Corynebacterium fascians* may occur in related diseases of other hosts, e.g. *Viola* sp. (Staniland & Goodey, 1934; Lacey, 1948), *Heuchera* sp. (Lacey, 1939) and *Scabiosa* sp. (Crosse & Pitcher, unpublished).

Although the strawberry diseases discussed in this paper are related to the joint presence of an eelworm and a bacterium, the possibility of the eelworm causing a disease by itself cannot be excluded. If such a disease does in fact exist under natural conditions it has not been identified during this investigation, nor does it seem likely that it can be produced experimentally until techniques are sufficiently improved to exclude *C. fascians* completely. Attention is now being directed therefore to the problem of eliminating contaminants from test plants, as well as from eelworms, by chemical sterilization and other means. With improved methods available it is hoped to determine the effect on strawberry plants of eelworms alone and to investigate critically red plant and other diseases allied to cauliflower.

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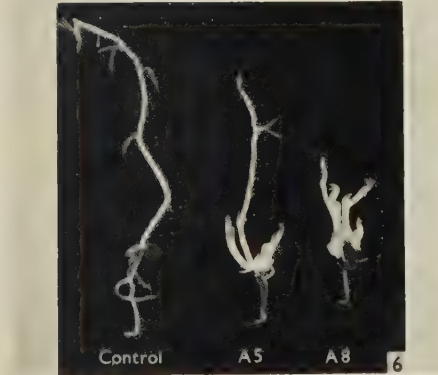
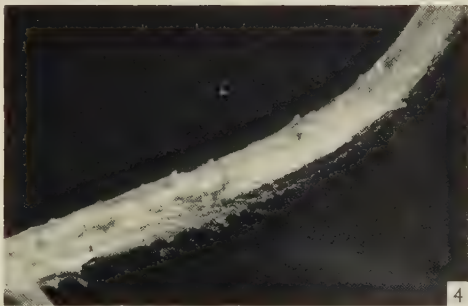
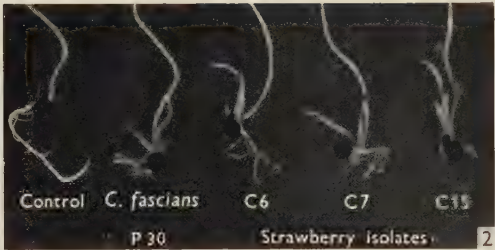
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EXPLANATION OF PLATE 17

- Fig. 1. Cauliflower crown on Mme Lefebvre strawberry.
- Fig. 2. Fasciation of sweet pea caused by three strawberry cauliflower isolates (C6, C7 and C15) compared with a sweet pea strain of *Corynebacterium fascians* (P30).
- Fig. 3. Feeding areas of *Aphelenchoides ritzema-bosi* on strawberry leaflets.
- Fig. 4. Strawberry pedicel bearing enations.
- Fig. 5. Group of alamate leaves in strawberry crown infected with *Aphelenchoides ritzema-bosi* and *Corynebacterium fascians*. The arrows indicate the petiole bases of leaves of normal size.
- Fig. 6. Fasciation of sweet pea caused by strains A5 and A8 of *Corynebacterium fascians* isolated from alamate leaves of field strawberries.

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CROSSE AND PITCHER—*Studies in the relationship of eelworms and bacteria to certain plant diseases*

RESOLUTION OF STRAWBERRY VIRUS COMPLEXES

V. EXPERIMENTS WITH VIRUSES 4 AND 5

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Strawberry virus 4 produces vein chlorosis and necrosis on strawberry (var. Royal Sovereign) and slight chlorotic spotting on wild strawberry (*Fragaria vesca* L.). No vector is known. Virus 5 produces leaf curling and vein necrosis on Royal Sovereign and *F. vesca*. It is transmitted by strawberry aphids (*Pentatrichopus fragaefolii* Cock.) which have fed on an infected plant for 1 hr. or more and persists for about 1 hr. in the vector.

The names strawberry mottle, mild yellow-edge, crinkle, vein chlorosis and leaf-curl virus are proposed for strawberry viruses 1, 2, 3, 4 and 5 respectively.

In addition to three viruses described previously (Prentice & Harris, 1946; Prentice, 1948, 1949; Prentice & Woollcombe, 1951), two others affecting strawberry plants have now been identified. Data on these are incomplete, but are put on record because the author has discontinued work on the problem.

VIRUS 4

In experiments during 1944 and 1945, slight vein-clearing developed on healthy strawberry plants (var. Royal Sovereign, M40 clone) to which strawberry aphids (*Pentatrichopus fragaefolii* Cock. = *Capitophorus fragariae* Theob.) had been transferred after feeding for 3 weeks or more on plants with yellow-edge. The vein-clearing, which was first noticed 3-4 weeks after the aphids were transferred, gradually developed into veinal chlorosis (or narrow yellow vein banding) on short lengths of the subsidiary veins. This vein-clearing and chlorosis have already been reported (Prentice, 1948).

TABLE I

Infector: Huxley (clones S25 and M44).

Infection feeding period of stock: 12 weeks.

Indicator: *Fragaria vesca* and Royal Sovereign (clone M40).

Infector	Indicator	Aphids per indicator	Plants infected	Symptoms
S25	<i>F. vesca</i>	10	5/5	Virus 1 (severe)
	Royal Sovereign	10	1/5	Veinal chlorosis
M44	<i>F. vesca</i>	10	5/5	Virus 1 (severe)
	<i>F. vesca</i>	20	5/5	Virus 1 (severe)
	Royal Sovereign	10	5/5	Veinal chlorosis
	Royal Sovereign	20	2/5 5/5	Definite chlorotic spotting

Vein chlorosis occurred also on Royal Sovereign plants colonized by aphids which had fed on Huxley strawberry plants (see Table 1).

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In this experiment the aphids on *Fragaria vesca* indicators were transferred to fresh *F. vesca* plants after 24 hr. Faint chlorotic spots developed on three of these; no symptoms were observed on the remainder. The possible significance of the faint spots was not appreciated at the time.

In another experiment, five aphids, after feeding on a Royal Sovereign plant with yellow-edge for 3 weeks, were transferred to each of five indicators for 24 hr. and then retransferred to fresh Royal Sovereign plants. One of the latter developed vein chlorosis, but the symptoms disappeared later. This plant was grafted to a *F. vesca* plant by the stolon inarching method (Harris & King, 1942), but no symptoms were observed on the *F. vesca* plant.

Efforts to retransmit the virus causing vein chlorosis were unsuccessful. Infection-feeding periods of up to 8 weeks were used and aphids were retransferred after a first transfer-feeding period of 1 or 4 hr. (by this means it was hoped to obtain the new virus free from contamination with virus 1, in the second or later transfer). Both *F. vesca* and Royal Sovereign were used as indicators. Symptoms of virus 1 infection developed on *F. vesca* indicators of the first transfer-feeding period; indefinite chlorotic spots developed on a few *F. vesca* indicators of the second and third transfers. A few Royal Sovereign plants were suspected of vein chlorosis, but when they were grafted to *F. vesca* plants, no symptoms were produced on the latter. Vein-clearing was too unreliable and too fleeting to use in diagnosis; vein chlorosis was definite in some plants but often faded, so that in a few weeks it was no longer discernible, and in others it was never more than very slight. It was therefore impossible to have complete confidence in diagnosis, and the small number of infections obtained in transmission experiments do not justify any definite conclusions.

In 1947, Mr J. Turnbull drew the author's attention to symptoms shown by commercial stocks of Royal Sovereign (M40 clone) in the field. Short lengths of the veins were chlorotic or, in some plants, necrotic. Veinal chlorosis was noticeable on the young leaves, chlorosis and necrosis on the older leaves. Infected plants were slightly reduced in vigour, and necrosis of portions of the main veins caused a characteristic backward bending of some leaflets (generally the terminal leaflet on one of the older leaves).

Plants showing these symptoms were transferred to the glasshouse, where the symptoms tended to disappear during the summer but reappeared later in the year. Affected plants were grafted to symptomless Royal Sovereign plants and produced veinal chlorosis on seven of them. The disease is therefore graft-transmissible and presumably caused by a virus. Other specimens were grafted to *F. vesca* plants, but, apart from slight evanescent flecks on the veins, no symptoms appeared. The responsible virus will be referred to as virus 4.

Attempts to transmit virus 4 by *Pentatrichopus fragaefolii* with infection-feeding periods of 6 hr., 24 hr., 4 days, 6 days and 14 days showed no results. The failure may be apparent only, because symptoms on Royal Sovereign are rather slight

and sometimes masked, while symptoms on *Fragaria vesca* are so slight as to be of little diagnostic value.

The virus previously mentioned as occurring in 1944 during experiments with *Pentatrichopus fragaefolii* vectors, and producing veinal chlorosis on Royal Sovereign, may be identical with virus 4. Very faint chlorotic spotting, apparently caused by a persistent virus, has also been noted from time to time in aphid-transmission experiments with *Fragaria vesca* indicators, and spotting of this kind is also caused by virus 4. The possibility that virus 4 is a persistent virus transmissible by *Pentatrichopus fragaefolii* is, therefore, worth further exploration.

VIRUS 5

In July 1946, aphids which had fed on an apparently symptomless plant of the American strawberry variety Fairfax for 14 days, were transferred to *Fragaria vesca* plants on which they remained for 24 hr. About 5 weeks later, these developed symptoms different from any previously described. Portions of the veins became chlorotic and then necrotic, and the leaflets were strongly downcurled and twisted. The vein necrosis spread until, 3 weeks later, the older leaves of some plants were dead. The plants quickly recovered and later appeared almost normal, although somewhat lacking in vigour. In the following May, slight symptoms of leaf-curling and vein necrosis reappeared. No symptoms were noticed in May 1948, but in October the plants lacked vigour and some of the leaves were slightly twisted.

TABLE 2. *Isolation of virus 5*

Infectior: Strawberry plant var. Fairfax.

Infection-feeding period: 14 days.

Indicator: *F. vesca*. Five aphids per indicator.

Transfer	Test feed	Plants infected
1	24 hr.	4/5
2	3 days	0/5

Using as a source one of the *F. vesca* plants infected in 1946, the virus was transmitted in May 1947 with an infection-feeding period of 24 hr. Eight aphids were transferred to each of four *F. vesca* plants, three of which became infected. The virus was also transmitted with infection-feeding periods of 4 and 12 days, using five aphids per indicator. Three plants out of ten became infected and symptoms appeared in 4-5 weeks.

An experiment was made in August 1947 to determine the shortest period of feeding by which aphids became infective (i.e. to determine the acquisition threshold period). A plant infected during the previous May was used as an infector, and results are given in Table 3, Exp. A.

Three similar experiments were made in May and June 1948 in which the infectors were *F. vesca* plants infected in 1946 or 1947. Infection-feeding periods were 1, 2, 3 and 24 hr., and 1, 2, 4 and 8 hr., and 1, 2, 6 and 24 hr., respectively, and five aphids were transferred to each indicator. No infections occurred, and

F. vesca plants that have been infected for some months and no longer show acute symptoms may be poor sources of virus.

The virus was re-isolated without difficulty from the original Fairfax plant (see Table 3, Exps. B and C). Further tests using recently infected *F. vesca* plants were then made with the results shown in Table 3, Exps. D and E.

Virus 5 can be transmitted after an infection-feeding period of 1 hr., but a higher proportion of aphids transmit with an infection feed of 24 hr.

To determine the persistence of the virus in the vector, aphids were allowed to feed on an infected plant for 24 hr. and then transferred singly to each of five indicators, either direct or after a period of 1, 2 or 3 hr. on a detached leaf of a healthy plant. Two of the five plants to which aphids were transferred direct became infected; none of the fifteen plants to which aphids were transferred after an intervening period became infected. In addition, five aphids were transferred to each of five indicators after 24 hr. on an infected plant and 3 hr. on a healthy

TABLE 3. *Transmission of virus 5 after different infection feeds*

Infectors: (Exps. A, D and E), *F. vesca* infected with virus 5; (Exps. B and C), Fairfax.
Indicator: *F. vesca*. Five aphids per indicator.

Infection feed (hr.)	Plants infected					Total
	Exp. A	Exp. B	Exp. C	Exp. D	Exp. E	
1	0/5	—	0/5	0/5	2/5	2/20
2	—	—	—	0/5	1/5	1/10
3	3/5	—	—	—	—	3/5
4	—	—	—	3/5	3/5	6/10
6	2/5	—	1/5	—	—	3/10
8	—	—	—	1/5	5/5	6/10
18	2/5	—	—	—	—	2/5
24	5/5	4/5	3/5	5/5	5/5	22/25
48	—	—	4/4	—	—	4/4

leaf. None of these indicators became infected. In another similar experiment, five aphids were transferred to each indicator. One of the five receiving aphids direct, and none of the fifteen receiving aphids which had passed an intervening period on a healthy leaf, became infected. It is concluded that, when the vector is feeding, the virus does not usually persist for more than 1 hr.

F. vesca plants grafted by the stolon-inarching method to the Fairfax plant from which virus 5 was isolated, became severely stunted, and the symptoms (described by Harris & King, 1942) were quite unlike those produced by virus 5. It seemed, therefore, that the Fairfax plant might contain another virus in addition to virus 5, but efforts to separate one were unsuccessful. With infection-feeding periods of 5 days, 3 weeks and 2 months, virus 5 was isolated in *F. vesca* indicators of the first transfer-feeding period. No symptoms were detected in indicators of serial transfers made after 24 hr.

Attempts were made to infect Royal Sovereign plants with virus 5, both by allowing infective aphids to feed on them and by grafting to infected *F. vesca*

plants. No definite symptoms were observed. The Royal Sovereign plants were planted outdoors to see if symptoms would develop, but because of the difficulty of maintaining outdoor plants free from aphids and the consequent risk of accidental transmission of a virus apparently new to this country, it was decided to destroy them after a few weeks, although no symptoms had appeared. Symptoms of leaf-curling and vein necrosis appeared later on infected Royal Sovereign plants in the glasshouse (T. M. Woollcombe, private communication).

DISCUSSION

For ease of reference while work was in progress, the viruses described in this series of papers were designated numerically. This system makes it easy to change a name if later work shows that what was at first considered to be a single virus is really a mixture. In general usage, however, descriptive names are preferred and the following are now proposed: virus 1, strawberry mottle virus; virus 2, strawberry mild yellow-edge virus; virus 3, strawberry crinkle virus; virus 4, strawberry vein chlorosis virus; virus 5, strawberry leaf-curl virus.

Virus 1 was tentatively equated with the mild crinkle virus by Prentice & Harris (1946), but the symptoms produced on Royal Sovereign do not, in the opinion of the present author, justify this assumption. At some seasons the symptoms of mild crinkle on Royal Sovereign are very slight and might be confused with those produced by virus 1 when the latter are at their greatest intensity. In early summer (June or July), however, the symptoms of mild crinkle are much more conspicuous; some of the lesions on the leaves become necrotic, and slight distortion or 'crinkling' of the leaves (from which the disease derives its name) is apparent. Necrosis and crinkling due to virus 1 has never been observed by the author, and it was for that reason that this virus was later considered to be distinct from mild crinkle and was named virus 1 (Prentice, 1948). The descriptive name, strawberry mottle virus, is now substituted for the numerical designation.

'Strawberry mild yellow-edge virus' is a cumbersome name, but it has already been applied to virus 2 (Prentice, 1948). It has been shown that this 'virus' is probably a mixture of viruses, however, and further work may therefore make renaming necessary.

The names proposed are based on the symptoms produced on a 'sensitive' variety of the cultivated strawberry, rather than on those produced on *F. vesca* which has been used as an indicator plant in the course of the work. The main observed characteristics of these viruses are summarized in Tables 4 and 5.

Mottle virus is common in cultivated varieties of the strawberry in England, but the symptoms produced are so slight that they do not usually constitute a diagnosable disease. Mild crinkle as diagnosed in the field is commonly caused by vein chlorosis virus or vein chlorosis and mottle viruses together. Severe crinkle is caused by the crinkle virus either alone or in combination with mottle and/or

vein chlorosis virus. Yellow-edge is caused by the mild yellow-edge virus in combination with mottle or crinkle virus.

So far as is known, leaf-curl virus does not occur in the field in Great Britain. The Fairfax plant from which it was transmitted was obtained from U.S.A. some years ago and has since been maintained in an insect-proof glasshouse at East Malling. It is presumed that the plant was infected when received. The symptoms of leaf-curl superficially resemble those of strawberry leaf-roll (Berkeley & Plakidas, 1942). The viruses are probably distinct, but further work is required to decide the question. The leaf-curl virus may be related to an unnamed virus mentioned by Smith & Moore (1952).

More viruses infect strawberry than was suspected when the work was begun, and circumstantial evidence suggests that still other viruses also infect this host.

Table 4 shows that the vector relations of the viruses studied are of three types: non-persistent, persistent without latent period, and persistent with latent period.

TABLE 4. *Vector relationships of strawberry viruses*

No. and name	Acquisition threshold period	Latent period	Persistence
1. Strawberry mottle	c. 1 hr.	None detected	Short (c. 1 hr.)
2. Strawberry mild yellow-edge	c. 24 hr.	None detected	Long
3. Strawberry crinkle	c. 24 hr.	c. 12-16 days	Long
4. Strawberry vein chlorosis	—	Vector not known	—
5. Strawberry leaf-curl	c. 1 hr.	None detected	Short

TABLE 5. *Symptoms produced by strawberry viruses*

Virus	Symptoms on <i>F. vesca</i>	Symptoms on Royal Sovereign
Mottle	Chlorotic spotting; leaf distortion	Faint chlorotic spotting
Mild yellow-edge	Slight chlorotic spotting; slight chlorosis; leaf-cupping	Slight chlorosis, mainly at margins of leaves
Crinkle	Severe chlorotic spotting; leaf distortion; vein necrosis	Chlorotic and necrotic spotting
Vein chlorosis	Faint chlorotic spotting	Vein chlorosis and necrosis
Leaf-curl	Leaf-curling; vein and leaf necrosis	Leaf-curling; vein necrosis

The mottle virus and the leaf-curl virus are non-persistent (in the terminology of Bawden (1950), but not necessarily of Watson & Roberts (1939)). The mild yellow-edge virus (or viruses) is persistent, but no latent period in the vector has been detected. The crinkle virus is persistent and has a long incubation period in the vector.

Bawden (1950, p. 83) has interpreted the data as implying a latent period of about 24 hr. for the mild yellow-edge virus; Mellor & Fitzpatrick (1951) consider also that a rather similar virus isolated by them from strawberry has a latent period. In several experiments with the mild yellow-edge virus, however (Prentice, 1948, Exps. 4, 5, 6 and 7), there was no evidence of any latent period. The absence of

infection in the first series of indicators in certain other experiments is not necessarily significant, because few infections were obtained in subsequent transfers. It is held, therefore, that no latent period has been detected. If one exists, it appears from Exp. 4 (Prentice, 1948) that it does not exceed 24 hr. (assuming the acquisition threshold period to be 24 hr.).

The latent period of the crinkle virus is so long that it is unlikely to be merely the time required for the virus to penetrate the insect gut wall and return to the salivary glands. If it is assumed that the delay is due to the small amount of virus ingested and the consequent small chance of virus reaching the salivary gland, it is difficult to see why infections occur frequently and regularly as soon as the latent period is passed. It may, of course, be that the passage of virus affects the gut so that the passage of further virus is rapid, or that the unit of virus crossing the wall is large enough to cause several infections. Neither hypothesis is particularly impressive.

Another possibility is that the long latent period is the time required for the ingested virus to multiply sufficiently to be transmitted to strawberry plants. Clover club-leaf virus has been shown to multiply in its vector (Black, 1950), but although the possibility with the strawberry crinkle virus cannot be ruled out, the available data do not support it. An aphid which feeds on an infected plant for 1 day can cause infection approximately 15 days afterwards (Prentice & Woollcombe, 1951). On the hypothesis of multiplication, therefore, the amount of virus ingested in 1 day presumably multiplies to an infective concentration in 15 days. If the aphid feeds on an infected plant for a longer period, the virus ingested on the second and later days will augment that ingested on the first day and will multiply also, so that the infective concentration will be reached sooner. The latent period (i.e. the observed delay plus the infection-feeding period) would thus be expected to decrease with increasing length of infection-feeding period. The available data are inconclusive, but suggest, on the contrary, that the latent period is slightly increased with increasing infection feed.

A more probable explanation for the latent period is that either the virus is non-infective as absorbed by the vector or is rendered so in the vector. The latent period would then represent the time taken for the release of the imbibed virus in an active form.

I am indebted to Miss T. M. Woollcombe for assistance in these experiments. I am also indebted to Dr R. V. Harris for advice and criticism; the opinions expressed in this paper are, however, entirely my own.

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STUDIES IN *RUBUS* VIRUS DISEASES

IV. YELLOWS DISEASES OF RASPBERRIES

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(With Plate 18)

Yellows diseases of raspberry, previously considered to be physiological disorders, have causes, presumably viruses, that are transmitted by grafting. Several can be distinguished by the severity of symptoms produced in different raspberry varieties, but whether they are caused by distinct viruses or by related strains is uncertain. Two, called mild and severe yellows, are thought not to be related to the yellow mosaic and yellows virus described in North America.

A brilliant yellowing of the foliage of the raspberry variety Devon was described by Harris (1933) under the name 'Devon chlorosis'. He attempted graft transmissions to Baumforth's Seedling B but without result (private communication). A similar yellowing of the varieties Norfolk Giant, St Walfried and Lloyd George was common in Scotland in 1945 and subsequent years, and in 1947 was transmitted by grafting. These tests revealed a new group of raspberry virus diseases. So far, two etiologically different diseases, provisionally named mild and severe yellows have been distinguished on Norfolk Giant. It is evident that, along with Devon chlorosis, which is almost certainly also a virus disease, there are others, though the etiological relations between these and mild and severe yellows are not yet known.

The techniques and terms used were described earlier (Cadman, 1951).

SYMPTOMS ON NORFOLK GIANT

Severe yellows symptoms are most conspicuous in early spring and show as a vivid yellowing or bronzing of the interveinal areas of the leaves of the fruiting laterals and lower leaves of the young canes (Pl. 18, fig. 2). In extreme cases, the interveinal tissue may become completely bleached and necrotic. On leaves produced later in the season, chlorosis is usually less intense and frequently forms a water-mark or oak-leaf pattern.

Mild yellows symptoms also are most clearly expressed on the leaves of the fruiting laterals and lower leaves of the young canes in early spring. Typically, the interveinal areas of the first formed leaves become pale yellow and may develop

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necrotic areas. On leaves produced later in the season, the chlorosis is confined to narrow bands bordering the smaller leaf veins (Pl. 18, fig. 1). Leaves produced in midsummer show only a diffuse chlorotic watermarking.

The symptoms of both diseases vary widely in intensity from one season to another for unknown reasons.

TRANSMISSION

Severe yellows

Severe yellows was first noticed in 1947 on a plant of Norfolk Giant which was infected with moderate vein chlorosis virus (Cadman, 1952*a*), and had been grafted the previous season to an apparently healthy plant of Burnetholm Seedling. The infected Norfolk Giant was grafted in 1947 and 1948 to four plants of L234 Norfolk Giant (infected with leaf mottle; Cadman, 1951), all of which developed symptoms of severe yellows and moderate vein chlorosis. These Norfolk Giant plants were used as infectors in a series of grafting experiments in 1949 and 1950. Healthy plants of St Walfried developed apical necrosis, rosetting of the terminal leaves and conspicuous yellow blotches 7 weeks after grafting. In the following spring the leaves of the fruiting laterals and lower leaves of the young canes were fringed with intense yellow (Pl. 18, fig. 3). The plants also showed symptoms of leaf mottle but not of moderate vein chlorosis. The three plants grafted in 1949 were grown in the glasshouse during 1950. Cane growth was not markedly inhibited and yellows symptoms, in the form of a pepper-and-salt pattern of yellow and white spots, showed on occasional leaves throughout the season.

Five plants of Lloyd George (New Zealand stock) developed vein chlorosis, diffuse leaf mottling and rosetting of shoot tips 4 weeks after grafting in July 1949. These plants were transplanted to open ground in autumn 1949 and all showed conspicuous yellowing in spring 1950 and in 1951. Leaf symptoms were similar to those described on Norfolk Giant and, compared with ungrafted control plants, the canes were fewer and smaller and the leaves were down-curved (Pl. 18, fig. 4). Symptoms were partially masked during the summer of 1950, only occasional leaves being tipped or fringed with chlorosis.

Four plants of Baumforth's Seedling B were successfully grafted with infected Norfolk Giant scions in 1950, but none of these showed yellows in spring, 1951. Inconclusive results were obtained with Newburgh and the Malling varieties Promise, Jewel, Notable and Seedling M, plants of which were grafted with infected Norfolk Giant in 1950. Severe yellows symptoms developed only on the plants of Malling Notable. The negative results with the other four varieties may be due to prior infection of the plants with mild yellows or a related virus (see also below, p. 498).

Mild yellows

Samples from a commercial stock of Norfolk Giant showing mild yellows were grafted to fifteen plants of this variety (L234 stock) in 1947 and to a further five

plants in 1948. Six of the first set and all five of the second showed mild yellows symptoms in the following spring. As the infected plants all showed similar symptoms they were used as sources of mild yellows in the grafting experiments made in the years 1948-50. Assessment of the results was hampered by the tendency of symptoms on all varieties to become masked, and symptom expression was particularly poor in the 1950 season. Norfolk Giant has so far proved the most useful indicator for this virus, though mild yellows symptoms are often extremely difficult to diagnose in the presence of mosaic 2 or other viruses attacking this variety.

Healthy plants of St Walfried grafted in 1948 and 1949 with infected Norfolk Giant scions showed diffuse interveinal chlorosis in the current season and developed symptoms of mild yellows and leaf mottle in the subsequent year. Yellows symptoms appeared first and were most conspicuous on the leaves of fruiting laterals as general pale chlorosis. Later in the season these plants were distinguishable from others infected with the leaf-mottle virus only because the chlorotic spots caused by the latter were more sharply defined (Pl. 18, fig. 5). Transmission of mild yellows to two of the 1948 St Walfried plants was confirmed by back grafts to Lloyd George (New Zealand stock) in 1950.

In 1948, eleven plants of Lloyd George (New Zealand stock) were grafted with infected Norfolk Giant, and four of these showed interveinal chlorosis the following spring. The symptoms consisted of a pepper-and-salt pattern of pale chlorotic spots most clearly visible on the first-formed leaves (Pl. 18, fig. 6). Under glass, symptoms were masked for the greater part of the season but reappeared on the tip leaves in late summer as diffuse interveinal chlorotic patches. Mild yellows was detected in two of these plants by back grafts to Norfolk Giant. The experiment was repeated with five plants of Lloyd George in 1949 and gave similar results. These plants were transferred to the open ground and grown on in 1950. Leaves of the fruiting laterals and the first leaves on the young canes showed pale yellow chlorosis in early spring. Symptoms were masked on foliage produced later in the season, and the plants showed only an inconspicuous interveinal mottle. Canes on these plants were fewer and less vigorous than those on either ungrafted controls or plants infected with leaf mottle.

The symptoms described above are very common in the field on degenerate stocks of Lloyd George. Out of a sample of twenty-nine canes from such plants grafted to healthy plants of Lloyd George (New Zealand stock) in 1948, nineteen produced what appeared to be mild yellows symptoms on the indicators. Three of the twenty-nine also produced mild yellows on Norfolk Giant, and these three and a further four samples produced yellows on St Walfried. On inspection in spring 1949, the parent stools of the Lloyd George canes all showed yellows on the foliage of the fruiting laterals and young canes.

Five plants of Baumforth's Seedling B grafted with infected Norfolk Giant in 1949 did not develop yellows in either 1950 or 1951. Confusing results were

obtained with Newburgh and the Malling varieties Promise, Jewel, Notable and Seedling M in experiments made in 1949 and 1950. Typical mild yellows symptoms developed only on the grafted plants of Malling Notable. Whereas the plants of Malling Jewel and Malling Seedling M used showed no yellows symptoms at the time of grafting, the Newburgh and Malling Promise plants all showed slight yellow spotting on the lower leaves of the young canes. Sister plants of all these indicators were grafted to Lloyd George in 1950, but the tests gave equivocal results. Failure to transmit both mild and severe yellows to Newburgh and the Malling varieties mentioned above may be due to prior infection of the plants with a related yellows disease, and evidence of this is being sought.

RELATIONS BETWEEN MILD AND SEVERE YELLOWS

In 1950, three plants of Norfolk Giant infected with mild yellows were grafted with Norfolk Giant scions infected with a mixture of severe yellows and vein chlorosis viruses. All three developed vein chlorosis, but none showed severe yellows in the current season. Two of the plants were over-wintered in the greenhouse, and these developed mild yellows and vein chlorosis in spring 1951. The third plant, over-wintered outdoors, showed severe yellows in spring 1951. Though admittedly inconclusive, these results suggest a mutual protective action and therefore that mild and severe yellows may be etiologically related diseases.

'DEVON' CHLOROSIS AND YELLOWS SYMPTOMS ON OTHER VARIETIES

No plant of the Devon variety has been found completely free either from yellows symptoms or from infection with mosaic 2 diseases (Cadman, 1952*b*). Proof that the former are caused by virus infection is afforded only by grafting experiments with Lloyd George and St Walfried.

In 1948 and 1949, nine plants of Lloyd George (New Zealand stock) were grafted with scions from affected Devon plants. Six of the indicators developed diffuse interveinal chlorosis and rosetting of shoot tips the same year. Four of the five plants grafted in 1949 were transferred to the open, and all showed conspicuous yellowing of the foliage and fruiting laterals and lower leaves of the young canes in 1950. They produced an average of two canes, and the leaves were down-curved and were greenish yellow throughout the season.

Five healthy St Walfried plants were also grafted in 1949 with scions from the Devon infectors. These developed interveinal chlorotic spots in the current year, and three of the plants showed interveinal yellow streaks on occasional leaves in spring 1950. These symptoms were distinct from those of either mild or severe yellows on this variety. Five plants each of Newburgh and Malling Promise, all showing slight yellow spotting on the lower leaves of the young canes, developed no additional symptoms after grafting in 1950 with affected Devon scions.

Individual plants of Lloyd George, Norfolk Giant, Malling Promise and Malling Exploit have been found showing yellows symptoms distinct from any described

above on these varieties. In each case the disease has proved to be graft-transmissible either to Lloyd George or St Walfried, but there is no information on the etiological relations of these diseases with mild and severe yellows. Yellows symptoms have also been found on commercial stocks of Malling Landmark and Bath's Perfection (syn. Marlborough), but no experiments have been carried out with these varieties.

CONCLUSIONS AND DISCUSSION

Although the yellows diseases on Norfolk Giant and other varieties described above have commonly been considered non-infectious disorders, the results of the foregoing grafting experiments demonstrate conclusively that they are virus diseases. The etiological relations between them are still obscure, but it seems likely that the two types distinguished on Norfolk Giant and provisionally named mild and severe yellows respectively, are caused by related strains of one virus.

There is as yet no evidence of relationship between the North American yellow mosaic described by Bennett (1932) and other workers and the yellows diseases described here. A characteristic of both mild and severe yellows is the strong tendency of symptoms to become masked on foliage produced during warm weather. Symptoms of yellow mosaic on Cumberland black raspberry (*Rubus occidentalis*) and King red raspberry (*R. strigosus*), on the other hand, are said not to be masked by warmth (Bennett, 1932). The behaviour of yellow mosaic on European raspberry varieties and of mild and severe yellows on North American varieties is unknown.

In the field, virus diseases of the yellows type are common and widely distributed. The variety Devon is now defunct in Scotland, largely because of yellows. A similar disease is prevalent in stocks of Bath's Perfection (syn. Marlborough) in both Britain and Scandinavia (Lihnell, 1949; Harris, private communication). Severe yellows occurs in commercial stocks of Lloyd George and, symptoms of mild yellows are almost ubiquitous in stocks of Lloyd George, St Walfried and Norfolk Giant. Quantitative data on cane growth and cropping of the Lloyd George plants referred to above were collected but are of doubtful value because the virus content of the yellows infector plants is unestablished. It seems likely that yellows diseases are economically important in certain varieties, notably Lloyd George, but a precise assessment of their significance must await the development of more adequate techniques of analysis.

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EXPLANATION OF PLATE 18

- Fig. 1. Leaf from Norfolk Giant plant naturally infected with mild yellows.
- Fig. 2. Severe yellows symptoms on Norfolk Giant graft infected from Burnetholm Seedling.
- Figs. 3, 4. Severe yellows symptoms on St Walfried and Lloyd George (New Zealand stock) respectively.
- Fig. 5. Leaf from St Walfried plant showing combined symptoms of leaf mottle and mild yellows.
- Fig. 6. Mild yellows symptoms on Lloyd George (New Zealand stock) 8 weeks after graft inoculation.

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CADMAN—*Studies in Rubus virus diseases. IV*

STUDIES IN *RUBUS* VIRUS DISEASES

V. EXPERIMENTS IN THE ANALYSIS OF LLOYD GEORGE DECLINE

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(With Plate 19)

Degenerate plants of the raspberry variety Lloyd George are usually infected with a complex of viruses that produces mosaic 2 diseases on Baumforth's Seedling B and Norfolk Giant. This complex comprises mild yellows and at least two other viruses, one of which, raspberry leaf-spot virus, is transmitted by the aphid, *Amphorophora rubi*. This virus produces characteristic symptoms on Baumforth's Seedling B, Burnetholm Seedling and Norfolk Giant, and it is carried without symptoms by St Walfried, Lloyd George and many other European and North American varieties. Both mild yellows and leaf spot viruses seem to be only subsidiary causes of raspberry degeneration.

Earlier papers (Cadman 1952 *a, b, c*) dealt with viruses that cause mosaic diseases common in stocks of raspberry varieties. These diseases are of three main types, yellows, vein chlorosis and veinbanding; they are caused by at least six viruses. Practical experience in the raising of healthy raspberry stocks has shown that certain varieties, notably Lloyd George, may decline rapidly in vigour and productivity even though free from the more virulent of these viruses. This suggests that still other viruses infect raspberries. Evidence that this is so was provided by Harris (1933, 1940) and by Prentice & Harris (1950), who found that many raspberry varieties were symptomless carriers of viruses that produced symptoms on the varieties Baumforth's Seedling B and Norfolk Giant. The characteristic leaf symptoms on these indicators were translucent, angular chlorotic spots, accompanied by varying degrees of leaf and cane malformation and, sometimes in Baumforth's B, by the death of the plant. To symptoms of this sort Harris (1940) gave the name mosaic 2; he distinguished two principal grades and named these mild and severe mosaic 2.

The work reported in the present paper was begun with the object of establishing the cause of so-called 'symptomless decline', particularly of the variety Lloyd George. Pl. 19, fig. 1, illustrates one of several plants showing this condition that were selected from four different Scottish commercial stocks in 1947. These plants were free from vein chlorosis and vein-banding viruses but were all infected with yellows virus. They were grafted in 1948 to healthy New Zealand Lloyd George

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plants which were planted out in 1949 in a randomized plot trial along with ungrafted control plants. By 1950 the grafted plants all resembled their infectors, and they produced significantly fewer and smaller canes than the ungrafted controls (Table 1). A parallel set of New Zealand Lloyd George plants grafted in 1949 with yellows-infected Norfolk Giant scions did not show these effects. It was therefore concluded that 'symptomless decline' of Lloyd George is a virus disease and that yellows virus is not the primary cause.

Attempts to sort out the viruses present in degenerate Lloyd George plants were hampered by the inadequacy of the only analytic technique available; that of grafting infected plants to a range of indicator hosts and observing the symptoms thereon. Experiments along these lines showed that apparently healthy plants of Lloyd George produced less severe diseases on Baumforth's B and Norfolk Giant than did degenerate plants. Their common component was a virus that produced the characteristic leaf-spot symptoms referred to above. This virus proved to be transmitted by the aphid *Amphorophora rubi*, and to be widespread among raspberry varieties. Lloyd George plants infected only with this virus were, however, indistinguishable from healthy plants.

TABLE 1. *Growth records from healthy and virus-infected New Zealand Lloyd George plants; five plants per treatment*

Year	Control		Mild yellows		'Symptomless decline'	
	Mean no. canes	Mean ht. (cm.)	Mean no. canes	Mean ht. (cm.)	Mean no. canes	Mean ht. (cm.)
1950	8	101	2	100	5	65
1951	20	132	15	117	14	88

S.E. diff. between means: cane no. ± 1.2 , cane ht. ± 6.9 .

Subsequently, using the variety St Walfried, it was found that, whereas degenerate plants of Lloyd George produced a severe leaf-blotching disease on this indicator, apparently healthy plants did not. This result suggested that 'symptomless decline' was associated with at least two other viruses besides yellows, and that the component expressed by St Walfried might be the most important. Support for this was provided by data from three Lloyd George plants, originally from a Scottish source, kindly given to the writer by Dr I. W. Prentice. All three were typical degenerate plants, but whereas they produced severe blotching on St Walfried they produced no symptoms on Norfolk Giant. Little more is known about this third component except that it differs from leaf mottle, a virus that is also expressed by St Walfried and carried by Norfolk Giant (Cadman, 1951).

At present therefore 'symptomless decline' of Lloyd George is known to be associated with three different viruses: yellows, described earlier (Cadman, 1952c) a virus transmitted by *Amphorophora rubi* which will be called leaf spot, and a third virus, on which work is incomplete. There may well be others. The purpose of

this paper is to present the results of work with the leaf-spot virus which is considered to cause some of the diseases formerly designated mosaic 2 by Harris (1940) and Prentice & Harris (1950).

The techniques and terms used were described earlier (Cadman, 1951).

SYMPTOMS OF LEAF SPOT ON NORFOLK GIANT

The following description is made from plants infected by means of *A. rubi*. The leaves are covered with translucent, angular chlorotic spots which frequently coalesce, resulting in curling, twisting and, often, asymmetric development of the lamina (Pl. 19, fig. 2). Cane growth may at times be characteristically 'throttled' or stunted, a check that induces the outgrowth of axillary branches. These symptoms are not masked in hot weather.

Transmission by grafting

Thirteen plants of Lloyd George from the same source as sample A of Prentice & Harris (1950) were grafted to healthy plants of Norfolk Giant and Baumforth's B in 1944. The Lloyd George plants were the most vigorous that could be found in the parent stock though all showed moderate vein chlorosis. They were combined in

TABLE 2. *Results of graft transmissions of leaf spot from St Walfried to other varieties, in 1947*

Indicator	No. leaf spot No. of plants grafted	Leaf spot in check tests
Baumforth's B	6/6	—
Norfolk Giant	23/61	—
Burnetholm Seedling	1/1	1/1
Malling Landmark	0/2	1/1
Malling Promise	0/1	1/1

graft units as follows: Norfolk Giant grafted to Lloyd George grafted to Baumforth's B. In ten of these sets both the Norfolk Giant and Baumforth's B plants developed symptoms; in two the Baumforth's B plants alone showed symptoms, and in one set both the indicators remained healthy. All the infected Norfolk Giant plants showed leaf spot and precisely similar symptoms showed on the Baumforth's B plants. Experiments in 1947 (Table 2) showed that St Walfried, Malling Landmark and Malling Promise are also symptomless carriers of leaf spot, whereas Burnetholm Seedling, when infected, shows characteristic symptoms.

Transmission by aphids

In 1945, colonies of *Amphorophora rubi* and *Doralis idaei* were fed for 18–20 days on some of the infected Lloyd George, Norfolk Giant and Baumforth's B plants from the 1944 grafting experiment. Several hundred *Amphorophora rubi* were subsequently transferred to each of eleven healthy Norfolk Giant plants, of which one that received aphids from a Lloyd George infector developed leaf spot symptoms five weeks later. No such symptoms developed on ten Norfolk Giant plants

that received *Doralis idaei* or on eight control plants. This result was confirmed in 1946 using Lloyd George infectors only (Cadman & Hill, 1947). Table 3 shows that leaf spot was transmitted solely by *Amphorophora rubi* and that the aphids required 13-72 hr. infection feeding. Subsequent experiments did not supply more precise information on vector relations. Much higher rates of transmission were, however, obtained when very young raspberry seedlings were used as test plants.

Although these data show that leaf spot is transmitted by *A. rubi* after feeding for 13 hr. or more on a source of infection they do not show whether the disease is caused by one or more viruses. In the absence of such information it is assumed that only one virus is involved.

TABLE 3. *Results of aphid transmissions of leaf spot virus from Lloyd George to Norfolk Giant*

Aphid species	I.F.P. various; T.F.P. 3 and 14 days (bulkcd); Aphids per plant ∞				
	I.F.P.				
	12 hr.	3 days	7 days	14 days	21 days
<i>A. rubi</i>	0/10	1/10	1/10	2/10	0/10
<i>D. idaei</i>	0/10	0/10	0/10	0/10	0/10

TABLE 4. *Results of grafting experiments with Lloyd George in 1944*

Infector	Indicator	No. of plants grafted	Symptoms				
			Vein chlorosis	Mild mosaic	Severe mosaic	Necrosis	No symptoms
Sample 1 (Scottish)	Baumforth's B	7	0	0	2	5	0
	Norfolk Giant	9	0	1	3	3	2
	St Walfried	8	0	0	0	4	4
Sample 2 (English)	Baumforth's B	5	0	1	0	3	1
	Norfolk Giant	5	1	0	1	2	1

DISEASE COMPLEXES INVOLVING LEAF SPOT

Lloyd George

The results of the aphid and graft transmissions, though mutually consistent, convey an over-simplified impression of the virus content of samples from commercial Lloyd George stocks. It was, in fact, unusual to find such samples infected only with leaf-spot virus. The results (Table 4) of experiments made in 1944 are more typical.

Canes from a Scottish commercial stock of Lloyd George were divided into three lots and grafted, respectively, to healthy plants of Baumforth's B, Norfolk Giant and St Walfried. Other canes, from the same source as sample C of Prentice & Harris (1950), were divided into two lots of five and grafted, respectively, to plants of Baumforth's B and Norfolk Giant. Some of the Baumforth's B plants in each experiment developed leaf-spot symptoms together with necrosis of the fruiting laterals and young canes; they died in 1945. Some of the Norfolk Giant indicators

showed typical leaf-spot symptoms; others showed intense chlorotic spotting, leaf distortion, incipient cane necrosis and pronounced branching. More than half the St Walfried plants showed severe leaf blotching, and some of the plants became necrotic. The Lloyd George infectors in both experiments showed only moderate vein chlorosis, and a diffuse mottle probably caused by yellows virus, though the plants were not vigorous and the leaves were down-curved and greasy.

Subsequent work with Lloyd George showed that, whereas plants infected with leaf-spot virus only were indistinguishable from healthy plants, the degenerate condition (Pl. 19, fig. 1) was always associated with viruses that produced intense symptoms on Norfolk Giant and severe leaf blotching and cane stunting on St Walfried. It was therefore concluded that leaf spot is not the primary cause of 'symptomless decline' of Lloyd George because it is carried without symptoms by both this variety and St Walfried.

Other varieties

Experiments were made in 1948 and 1949 with vigorous plants of Newburgh and the Malling varieties Enterprise, Jewel, Notable, Promise and Seedling M selected from the best available stocks. They were grafted to parallel sets of healthy plants of Baumforth's B, Norfolk Giant and St Walfried. All the plants proved to be virus-infected and produced on all three indicators diseases analogous to those observed in the Lloyd George experiments described above (Pl. 19, fig. 3). The behaviour of St Walfried was correlated with that of the other two indicators. Infectors that produced severe leaf blotching and necrosis on St Walfried also produced severe or lethal diseases on Norfolk Giant and Baumforth's B; whereas those that produced mild leaf blotching or no symptoms on St Walfried produced diseases of only moderate severity on the other two indicators. Plants of Malling Promise and Seedling M were found that produced no symptoms on St Walfried or Norfolk Giant; they were, however, indistinguishable from the virus-infected plants referred to above. No uninfected plants of the remaining varieties were found.

The appearance of the infected Norfolk Giant and Baumforth's B plants in these experiments suggested that the basic symptoms were those of leaf spot, and that the more severe diseases were caused by leaf spot in conjunction with other viruses, the identity of which could only be guessed at. Severe diseases of Norfolk Giant, analogous to those found in the 1948 and 1949 experiments, however, were produced in 1951 by infecting healthy plants simultaneously with leaf-spot and mild yellows viruses, and with these two plus a third virus found in degenerate Lloyd George plants.

During the years 1944-9 an extensive search was made for healthy stocks of raspberry varieties. The criterion of health was freedom from viruses that produced mosaic 2 symptoms (*sensu* Harris, 1940) on Norfolk Giant. No attempt was made to analyse the diseases recorded in these routine tests. The results shown in Table 5

TABLE 5. *Results of grafts of stocks and varieties of raspberries to Norfolk Giant*

Infector	Origin	No. of samples	Year of graft	No. of indicators grafted	No. of grafts failed	No. of indicators showing severe mosaic 2
Wild raspberries	Scotland	25	1945	43	10	27/43
Lloyd George	England	2	1944, 1946	40	0	27/40
Lloyd George	Scotland (comm.)	26	1945, 1946	140	35	81/140
Lloyd George	Scotland (garden)	4	1946	35	4	30/34
Lloyd George	Scotland (garden)	2	1946, 1947	105	0	47/105
Lloyd George	New Zealand	1	1946	94	0	0/94
Lloyd George	New Zealand	7	1949	26	0	4/26
Lloyd George	New South Wales	1	1946	3	1	2/3
Devon	Scotland	2	1945, 1947	14	3	11/14
Malling Seedling G	Scotland	1	1948	10	4	5/10
Malling Enterprise	England	3	1947, 1948	61	6	54/61
Malling Jewel	England	2	1947, 1948	47	8	39/47
Malling Jewel	Scotland	1	1948	12	3	9/12
Malling Seedling K	Scotland	1	1949	25	0	19/25
Malling Landmark	Scotland	3	1947, 1949	59	0	0/59
Malling Seedling M	Scotland	2	1947, 1948	43	0	15/43
Malling Notable	Scotland	2	1947, 1948	29	0	29/29
Malling Promise	Scotland	3	1947, 1949	90	0	3/90
Malling Seedling V	Scotland	1	1948	12	1	8/12
Malling Seedling W	Scotland	1	1948	25	1	23/25
Malling Exploit	Scotland	2	1948, 1949	60	0	5/60
Malling Seedling Z	Scotland	1	1948	12	0	7/12
Mitchell's Seedling	Scotland	1	1945	12	3	9/12
Preussen	Scotland	1	1945	5	0	5/5
Preussen	New Zealand	1	1949	1	0	0/1
Red Cross	Scotland	1	1945	9	0	9/9
Gertrudis	Holland	1	1949	2	0	2/2
Radboud	Holland	1	1949	2	0	1/2
Chief*	New Zealand	1	1949	2	0	2/2
Cuthbert	England	1	1945	3	0	3/3
Cuthbert*	New Zealand	1	1949	2	0	0/2
Latham*	New Zealand	2	1949	3	0	3/3
Marcy	New Zealand	1	1949	1	0	1/1
Marlborough	England	1	1947	3	0	3/3
Newburgh	Scotland	1	1945	9	4	5/9
Newburgh	England	1	1947	25	6	19/25
Newburgh	New Zealand	1	1949	2	0	2/2
Newman	New Zealand	1	1949	2	0	2/2
Viking*	New Zealand	3	1949	5	0	1/5

* As the nomenclature of New Zealand stocks and varieties is confused, the identification of these samples is uncertain. The sample of Cuthbert was grown under the name Red Imperial; one sample of Latham grown as Cuthbert; and two samples of Viking as Admiral Byrd. The sample of Chief was unnamed.

can, therefore, only be held to show that both wild and many cultivated varieties of raspberries in Britain behave as symptomless carriers of viruses that produce mosaic 2 symptoms on Norfolk Giant, and that the same or related viruses occur in Holland, Australia and New Zealand. One set of samples of the varieties Chief,

Latham, Marcy, Newburgh, Newman 23 and Viking came from New Zealand. As *Amphorophora rubi* has not yet been recorded there (Cadman, 1948) it seems likely that the stocks were infected before they were imported from North America. Cadman (1952*a, b*) reported that, when Canadian plants of Latham, Viking and Cuthbert were grafted with Norfolk Giant scions, the scions developed mosaic 2 symptoms. Only two out of forty samples from vigorous stocks of Lloyd George in Great Britain were free from virus infection in these tests. Both were from garden stocks grown in the north of Scotland. By contrast, seven out of nine New Zealand stocks were free. A batch of ninety-four canes from a stock grown in the Tadmor district of Nelson province produced no symptoms on Norfolk Giant when tested at Dundee in 1946. These formed the nucleus of a new stock now in circulation under the name New Zealand Lloyd George.

The results with Mitchell's Seedling, Preussen, Red Cross and Marlborough (syn. Bath's Perfection) confirm those of Harris (1940).

DISCUSSION

The displacement of the established name mosaic 2 in favour of yet another new raspberry virus name calls for an explanation. Harris (1940) divided raspberry mosaic symptoms into two etiologically distinct groups, mosaic 1 and mosaic 2. The symptoms designated mosaic 2 were shown only by Baumforth's B and could be subdivided into two groups defined as 'mild mosaic 2 with slight or evanescent type *c* symptoms (angular chlorotic spots) and undetectable or very slow decline of the indicator; (ii) severe mosaic 2 with intense and concentrated type *c* leaf symptoms and a rapid decline and death of the indicator'. The results of grafting experiments quoted in support of these conclusions show that variations in symptoms classed as severe mosaic 2 were also found. References to severe mosaic 2 in a subsequent paper (Prentice & Harris, 1950), however, suggest that this name denoted only one disease.

The diseases described in the present paper may all be considered to belong to the subgroup severe mosaic 2 as originally defined. There is little doubt that they are etiologically different, though one component is evidently common to all. This component is a virus that is transmitted by *A. rubi* and produces a severe but not lethal disease on Baumforth's B and Norfolk Giant; it is carried without symptoms by Lloyd George and St Walfried. To avoid further misconceptions it seems better to name this leaf-spot virus rather than to equate it with the cause of severe mosaic 2 disease.

A disease conforming with the definition of mild mosaic 2 was widely found in commercial stocks of Norfolk Giant and also in wild raspberries in Scotland. It was graft-transmissible to Norfolk Giant and Baumforth's B, but its relationship to the leaf-spot virus is unknown.

Harris (1940) suggested that mild and severe mosaic 2 diseases were analogous to the North American green mottle mosaic. However, green mottle mosaic

symptoms according to Cooley (1936) are masked in hot weather whereas mosaic 2 symptoms are not. Moreover, all the North American red raspberry varieties examined have proved to be infected with viruses that produce mosaic 2 symptoms on Norfolk Giant. The absence from the literature of any parallel results suggest that the occurrence of such viruses in North America has been overlooked.

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EXPLANATION OF PLATE 19

- Fig. 1. Plants of New Zealand Lloyd George planted outdoors in autumn 1949; photographed June 1950. On right, ungrafted control; on left, plant grafted to degenerate Lloyd George infector in 1948.
- Fig. 2. Leaf from Norfolk Giant plant infected with leaf-spot virus by means of *Amphorophora rubi*, 1946. (Reproduced, by permission, from *J. hort. Sci.* **25**, 125.)
- Fig. 3. Complex symptoms on a Norfolk Giant plant grafted to an apparently healthy plant of Malling Promise.

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CADMAN—*Studies in Rubus virus diseases. V*

THE INFLUENCE OF PLANTING DATE AND MANURING ON THE INCIDENCE OF VIRUS DISEASES IN POTATO CROPS

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(With 1 Text-figure)

Field experiments with Majestic potatoes were made over six years at Rothamsted to test the effects of varying date of planting and manuring on the yield of tubers and the incidence of the aphid-transmitted leaf roll and Y (rugose mosaic) viruses. Yield was increased by early planting, and by all the manures, especially dung. Early planting also usually increased the incidence of virus disease. Different manures had different effects on disease incidence; the average results from all comparisons showed the largest increase in incidence of both viruses from the use of dung; sulphate of ammonia increased the incidence of leaf roll, and muriate of potash that of rugose mosaic. Counts in two years showed that aphid populations were highest on the earlier planted potatoes, and were increased by dung, sulphate of ammonia and superphosphate, but were reduced by muriate of potash.

INTRODUCTION

Differences in planting date and manuring might be expected to influence the incidence of the aphid-transmitted viruses causing leaf roll and rugose mosaic (potato virus Y) by affecting the susceptibility to infection of healthy plants, the amount and distribution of virus in infected plants, and the number, form and mobility of the aphids infesting the crop. Effects are likely to vary with season, climate, soil and variety of potato, and this may explain the conflicting results of previous workers, especially on the influence of manures (Janssen, 1929; Ross, Chucka & Hawkins, 1947; Hofferbert & Orth, 1948; Hofferbert, 1948; Bode, 1949; Bawden & Kassanis, 1950; Klapp, 1951; Krüger, 1951). Little work on the date of planting has been reported, but Czerwinski (1943) and Doncaster & Gregory (1948) found that crops colonized by aphids early showed a higher incidence of disease than those colonized later.

In each year from 1945 to 1950 experiments at Rothamsted, using main-crop potatoes (variety Majestic, Stock Seed Certificate), tested the influence of date of planting and manurial treatment on the incidence of leaf roll and rugose mosaic.

The standard form of the experiment consisted of four plantings spaced at intervals of about 3 weeks, each testing dung (D), nitrogen (N), phosphate (P), and potash (K) in all combinations. There were thus sixty-four treatments which were confounded into blocks of sixteen without replication. In 1945 and 1947 certain of the factors were omitted. The yields are not given in this paper because

they have been summarized in the *Rothamsted Experimental Station Report for 1950 (1951)*.

To study the increase of leaf roll and rugose mosaic within the plots single infected tubers were planted in known positions in each plot. The five plants on each side of these 'infectors' were sampled at the end of each season by taking one tuber from each plant ('infector unit' sampling). These tubers were planted the following year and the diseased plants were counted. In 1945 and 1946 samples were taken also in mid-season. For analysis the percentages of diseased plants for each treatment were transformed to degrees, and the results are given in degrees (Tables 1-6), so that the standard errors can be quoted. The values in degrees are of the same order of magnitude as the original percentages, and if the percentages are required they can be obtained by reference to Fisher & Yates (1938, Table XII).

To study the aphid vectors sticky insect traps (Broadbent, Doncaster, Hull & Watson, 1948) were operated near the plots each year, except in 1945 when the nearest trap was $\frac{1}{2}$ mile distant. Alate *Myzus persicae* (Sulzer) are thought to be the principal vectors of leaf-roll virus and virus Y in the area (Broadbent, 1950; Broadbent & Tinsley, 1951), and trap catches of this species only are shown in Fig. 1. The incidence of *Aphis rhamni* (Fonsc.), *Macrosiphum euphorbiae* (Thomas) and *Aulacorthum solani* (Kalt.) on the plants is mentioned in the text. Detailed aphid counts on the foliage were made only in 1948 and 1949.

1945 EXPERIMENT

The experiment was laid out in a 2^5 design in four blocks of eight plots, and certain interactions were confounded with block differences. Each plot of $\frac{1}{40}$ acre comprised eight rows by forty-five plants. In each plot the 15th plant of row 4 was a rugose-mosaic 'infector', and the 30th plant of row 5 was a leaf-roll 'infector'. Tubers were dug on 4 August and 1 October. The treatments were:

Planting date	Seed	Manuring
(1) 30 March	$\left\{ \begin{array}{l} \text{Sprouted in trays} \\ \text{Planted from bags} \end{array} \right\} \times$	(1a) No dung
(2) 20 April		(1b) Dung at 15 tons per acre at first planting, adjusted for changes in weight at subsequent plantings
(3) 11 May		(2a) No sulphate of ammonia
(4) 1 June		(2b) Sulphate of ammonia at 0.6 cwt. N/acre

A basal dressing of superphosphate and of muriate of potash was applied to all plots. In spite of this, however, the plots of the first planting without dung showed severe symptoms of potash deficiency at the end of July, and the haulms died early.

Aphids. *Myzus persicae* was not numerous, and few *Aphis rhamni*, *Macrosiphum euphorbiae* or *Aulacorthum solani* were found on the plants during the season. Trap catches (Fig. 1) on another part of the farm showed that some *Myzus persicae* were flying during April, May and June, and more during the last week of June and the

first two weeks of July when they were leaving the crop (data from Doncaster & Gregory, 1948).

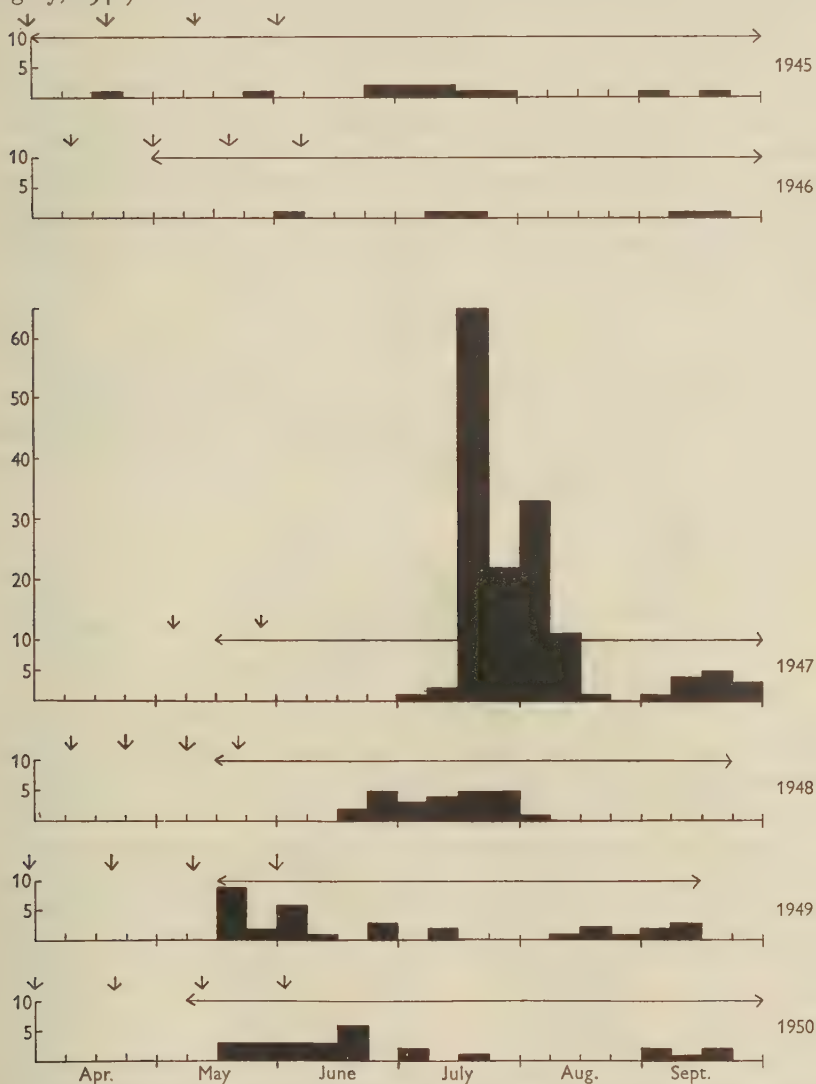


Fig. 1. *Myzus persicae* trapped in Rothamsted potato fields, 1945-50 (trap height 5-6 ft.).
↔ trapping period; ↓ planting dates.

Leaf-roll and rugose mosaic. Tubers dug on 4 August had a mean disease incidence for all treatments of 33 % leaf-roll and 8 % rugose mosaic. The incidence of the two diseases was 49 and 14 % respectively in tubers dug on 1 October. The

analysis after angular transformation is given in Table 1. The incidence of leaf roll in the October samples increased with each successive planting date, the differences between the first, second and third dates being significant.

In the August sample there was significantly more rugose mosaic in the second and third plantings than in the first and fourth. These differences were non-significant in the other sample. Applying D or N usually increased leaf roll, and did so significantly in the third planting. D also gave a barely significant increase in rugose mosaic at the first planting. D and N together increased both diseases, but only the leaf roll increase was significant. The only significant effects of sprouting in trays were to increase leaf roll in the first planting, and to decrease leaf roll in the third and rugose mosaic in the fourth plantings.

1946 EXPERIMENT

The experiment was in eight randomized blocks of eight plots each, and certain high-order interactions were confounded with block differences. Each plot was $\frac{1}{48}$ acre, six rows by forty-eight plants. Infected tubers were planted in position 16 in row 3, and position 32 in row 4 of each plot. Tubers were dug on 7 August and 8 October. The treatments were:

Planting date		Manuring
(1) 10 April	. ×	(1a) No dung
(2) 30 April		(1b) Dung as in 1945 experiment
(3) 20 May		(2a) No sulphate of ammonia
(4) 7 June		(2b) Sulphate of ammonia as in 1945 experiment
		(3a) No superphosphate
		(3b) Superphosphate, 0.6 cwt. P_2O_5 per acre
		(4a) No muriate of potash
		(4b) Muriate of potash, 1.0 cwt. K_2O per acre

Aphids. Potato aphids were fewer than in the other years in 1946. A few alate *Myzus persicae* and *Aphis rhamni* were founding colonies on the plants in early June. On 12 July (at the peak of infestation on other plots) a count on a small sample was made by taking nine leaves per plot from nine plants at random in the centre row, 576 leaves in all. The total aphids recorded were: 230 *Myzus persicae*, 130 *Aphis rhamni*, 23 *Macrosiphum euphorbiae* and 4 *Aulacorthum solani*. Even this small sample showed more aphids on the early plantings, total numbers being:

Planting date	<i>M. persicae</i>	<i>A. rhamni</i>
10 April	103	53
30 April	44	59
20 May	43	13
7 June	40	5

Leaf roll and rugose mosaic. Tubers dug on 7 August had a mean disease incidence for all treatments of 8.5 % leaf roll and 1.3 % rugose mosaic. The incidence was 11 % leaf roll and 1.5 % rugose mosaic in samples lifted on 8 October. The only significant effects of planting date were to increase leaf roll in the first planting and rugose mosaic in the second (Table 2).

TABLE 1. 1945 experiment (percentages transformed to degrees)

Incidence of leaf roll						
Plantings						
		1	2	3	4	Mean
August sample						
Mean	...	27.0	27.0	42.6	44.6	35.3
October sample						
Mean	(± 3.56)	27.0	39.6	51.3	58.9	44.2
No D	(± 5.04)	27.7	37.2	41.9	54.0	40.2
D		26.2	42.0	60.6	63.8	48.2
Response to D	(± 7.12)	-1.5	4.8	18.7	9.8	8.0*
No N	(± 5.04)	23.1	39.0	41.8	58.6	40.6
N		30.8	40.4	60.7	59.2	47.8
Response to N	(± 7.12)	7.7	1.4	18.9	0.6	7.2*
Not sprouted	(± 5.04)	15.0	41.8	59.2	59.2	43.8
Sprouted		39.0	37.4	43.4	58.6	44.6
Response to sprouting	(± 7.12)	24.0	-4.4	-15.8	-0.6	0.8*

* S.E. ± 3.56 .Responses to manures and sprouting (October sample) (± 5.04)

	D		N		Sprouting	
Response to	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D	.	.	-1.1	16.9	13.6	2.2
N	-1.9	16.1	.	.	9.0	5.2
Sprouting	6.5	-4.9	2.7	-1.1	.	.

Incidence of rugose mosaic

Plantings						
		1	2	3	4	Mean
August sample						
Mean	...	10.0	22.3	21.0	11.2	16.1
October sample						
Mean	(± 3.40)	20.0	24.4	23.9	19.9	22.0
No D	(± 4.81)	13.8	22.5	19.6	21.9	19.4
D		26.2	26.2	28.2	17.9	24.6
Response to D	(± 6.81)	12.4	3.7	8.6	-4.0	5.2*
No N	(± 4.81)	17.5	26.2	21.6	15.8	20.3
N		22.5	22.5	26.2	24.0	23.8
Response to N	(± 6.81)	5.0	-3.7	4.6	8.2	3.5*
Not sprouted	(± 4.81)	15.8	24.6	26.2	30.6	24.3
Sprouted		24.2	24.2	21.6	9.2	19.8
Response to sprouting	(± 6.81)	8.4	-0.4	-4.6	-21.4	-4.5*

* S.E. ± 3.40 .Responses to manures and sprouting (October sample) (± 4.81)

	D		N		Sprouting	
Response to	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D	.	.	11.8	-1.4	2.6	7.8
N	10.1	-3.1	.	.	6.5	0.5
Sprouting	-7.1	-1.9	-1.5	-7.5	.	.

TABLE 2. 1946 experiment (percentages transformed to degrees)

Incidence of leaf roll							
Plantings							
		1 2 3 4				Mean	
August sample							
Mean	...	21.2	12.2	15.4	18.1	16.7	
October sample							
Mean	(± 2.65)	29.1	13.5	16.7	17.4	19.2	
No D	(+3.74)	25.4	13.6	15.6	15.2	17.5	
D		32.8	13.3	17.8	19.6	20.9	
Response to D	(± 5.29)	7.4	-0.3	2.2	4.4	3.4*	
No N	(± 3.74)	29.2	15.8	14.2	17.6	19.2	
N		28.9	11.1	19.3	17.2	19.1	
Response to N	(± 5.29)	-0.3	-4.7	5.1	-0.4	-0.1*	
No P	(± 3.74)	32.4	14.1	20.4	18.0	21.2	
P		25.8	12.9	13.1	16.7	17.1	
Response to P	(± 5.29)	-6.6	-1.2	-7.3	-1.3	-4.1*	
No K	(± 3.74)	29.0	13.5	15.4	22.3	20.1	
K		29.1	13.4	18.1	12.5	18.3	
Response to K	(± 5.29)	0.1	-0.1	2.7	-9.8	-1.8*	

* S.E. ± 2.65 .

Responses to manures (± 3.74)								
Response to	D		N		P		K	
	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D	.	.	3.0	3.8	6.4	0.4	6.7	0.1
N	-0.5	0.3	.	.	0.7	-0.9	3.9	-4.1
P	-1.1	-7.1	-3.3	-4.9	.	.	-0.8	-7.4
K	-4.4	0.8	2.2	-5.8	1.5	-5.1	.	.

Incidence of rugose mosaic

		Plantings				
		1	2	3	4	Mean
		August sample				
	Mean ...	2.5	8.7	6.1	7.6	6.2
		October sample				
Mean	(± 1.36)	2.5	14.1	4.8	6.1	6.9
No D	(± 1.93)	0	10.1	1.6	5.7	4.3
D		5.0	18.1	8.0	6.6	9.4
Response to D	(± 2.73)	5.0	8.0	6.4	0.9	5.1*
No N	(± 1.93)	1.6	16.6	6.3	4.8	7.3
N		3.4	11.7	3.4	7.3	6.4
Response to N	(± 2.73)	1.8	-4.9	-2.9	2.5	-0.9*
No P	(± 1.93)	1.7	13.6	5.0	10.6	7.7
P		3.3	14.6	4.7	1.6	6.0
Response to P	(± 2.73)	1.6	1.0	-0.3	-9.0	-1.7*
No K	(± 1.93)	5.0	11.4	4.1	4.9	6.3
K		0	16.9	5.6	7.3	7.4
Response to K	(± 2.73)	-5.0	5.5	1.5	2.4	1.1*

* S.E. ± 1.36 .

Responses to manures (± 1.93)								
Response to	D		N		P		K	
	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D	.	.	4.6	5.6	3.6	6.6	9.4	0.8
N	-1.4	-0.4	.	.	1.5	-3.3	-0.4	-1.4
P	-3.2	-0.2	0.7	-4.1	.	.	2.0	-5.4
K	5.4	-3.2	1.6	0.6	4.8	-2.6	.	.

Manuring had no significant effects on the incidence of leaf roll. Rugose mosaic was significantly increased by D in the second and third plantings, and reduced by P in the fourth. Moreover, rugose mosaic was significantly increased by both D and P in the absence of K, and by K in the absence of D and P.

1947 EXPERIMENT

The severe winter and late spring of 1947 prevented early planting, and the experiment was reduced to four randomized blocks each of eight plots. There were two planting dates: 5 and 27 May. The effect of P was not tested, and all plots were given a basal dressing of superphosphate. Tubers were dug only at the end of the season (10 October).

Aphids. *Macrosiphum euphorbiae* and *Aulocarthum solani* were scarce, but large colonies of *Aphis rhamni* developed in late July and early August. *Myzus persicae* was scarce until mid-July when field observations and trap records (Fig. 1) showed the arrival of many alatae which evidently brought in leaf-roll virus. During the second half of July the population increased rapidly, and the summer dispersal migration from the potatoes occurred during the last few days of July and the first half of August, after which aphids were few until the middle of September, when the population again increased.

Leaf roll and rugose mosaic. The mean incidence of disease in the October samples was 71 % leaf roll and 14 % rugose mosaic. There was significantly more leaf roll in the later planted than in the early planted plots, but there was no difference in the incidence of rugose mosaic (Table 3).

In 1947, unlike other seasons in this series of experiments, the spread of leaf roll from the original 'infectors' was obscured by randomly distributed infections brought into the crop by alate *Myzus persicae* in July. Both D and N significantly increased leaf roll from 60 to 82 %. Manurial treatments had little effect on the spread of rugose mosaic, though N and K tended to reduce spread, significantly so when they were applied together.

1948 EXPERIMENT

The manurial treatments in 1948 were the same as in 1946, arranged in four randomized blocks of sixteen plots each. Planting dates were:

(1) 10 April, (2) 24 April, (3) 8 May, (4) 22 May.

Tuber samples were taken only on 28 September.

Aphids. There was a small spring migration of *Myzus persicae* and *Aphis rhamni* at the end of May, though no *Myzus persicae* were trapped during May or early June. *Myzus persicae*, *Aphis rhamni* and *Macrosiphum euphorbiae* were widely distributed over the plots, but were not very numerous during July, and the summer migration of *Myzus persicae* extended over seven weeks (Fig. 1).

The aphids on the foliage were counted on 21-22 July, coinciding with the maximum population of *Myzus persicae* and *Aphis rhamni*, and a week before the

maximum population of *Macrosiphum euphorbiae*. Broadbent (1948) and Anscombe (1948) showed that the number of aphids per plant was a more reliable way of expressing the population than the number per 100 leaves previously adopted. Their method was therefore used in expressing the counts of aphids per plot. The number of aphids was counted on one leaf taken at random from the top, middle and bottom regions of each of ten plants selected at random from rows 3 and

TABLE 3. 1947 experiment (percentages transformed to degrees)

		Incidence of						
		Leaf roll			Rugose mosaic			
		Plantings		Mean	Plantings		Mean	
		1	2		1	2		
Mean	(± 2.94)	49.8	65.0	57.4	(± 2.07)	21.2	22.4	21.8
No D	(± 4.16)	42.0	57.8	49.9	(± 2.93)	19.8	22.4	21.1
D		57.6	72.2	64.9		22.5	22.3	22.4
Response to D	(± 5.89)	15.6	14.4	15.0*	(± 4.15)	2.7	-0.1	1.3†
No N	(± 4.16)	41.4	59.8	50.6	(± 2.93)	24.5	23.0	23.8
N		58.2	70.1	64.2		17.8	21.7	19.7
Response to N	(± 5.89)	16.8	10.3	13.6*	(± 4.15)	-6.7	-1.3	-4.1†
No K	(± 4.16)	46.9	60.6	53.8	(± 2.93)	24.4	24.5	24.5
K		52.6	69.4	61.0		17.8	20.2	19.0
Response to K	(± 5.89)	5.7	8.8	7.2*	(± 4.15)	-6.6	-4.3	-5.5†

* S.E. ± 4.16 .† S.E. ± 2.93 .

		Responses to manures											
		(± 5.89)						(± 4.15)					
		D		N		K		D		N		K	
Response to		Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D		.	.	18.8	11.4	8.4	21.8	.	.	5.0	-2.2	0.6	2.2
N		17.3	9.9	.	.	19.7	7.5	-0.4	-7.6	.	.	2.8	-10.8
K		0.6	14.0	13.4	1.2	.	.	-6.2	-4.6	1.4	-12.2	.	.

4 of each plot. In addition, the total numbers of leaves on two plants per plot were counted to give both an estimate of plant size and data for expressing results on the basis of the number of aphids per plant.

The following sets of data were analysed statistically after applying a logarithmic transformation:

- (1) total aphids;
- (2) total alate aphids;
- (3) *Myzus persicae*;
- (4) *Macrosiphum euphorbiae*;
- (5) *Aphis rhamni*.

Table 5 summarizes the mean effects on the aphid populations of the different planting dates and manurial treatments. For all sets of data (with some inconsistency in *Aphis rhamni*) the earlier the potatoes were planted the higher the aphid

population. Again except for *A. rhamni*, P significantly increased the numbers of aphids. N and D also did so, but not always significantly. K, on the other hand, reduced numbers, the total number and *Macrosiphum euphorbiae* significantly so. Interactions of fertilizers with planting date showed that D or N applied to early planted plots significantly increased *Myzus persicae*, *Macrosiphum euphorbiae*, and total aphids. Total numbers of alatae were increased by N applied to early planted plots. K and P showed no significant interactions.

Leaf roll and rugose mosaic. The mean incidence of disease in the September samples was 28 % leaf roll and 11.5 % rugose mosaic. Planting date had no significant effect on either disease, despite the differences in aphid population on the plots planted on different dates (Table 4).

Leaf roll was increased by D on all planting dates, though not always significantly. Leaf roll was also significantly increased by N in the first planting. There were no significant responses to P or K. The only significant fertilizer interactions were to N+P which increased leaf roll, and to N+K which increased rugose mosaic.

1949 EXPERIMENT

The design of the experiment and the manurial treatments were the same as in 1948, but the rows contained 52 plants. Four infected tubers, two leaf roll and two rugose mosaic, were planted in each plot. In 1949 and 1950 the dung was ploughed in before the first planting. Planting dates were:

(1) 29 March, (2) 20 April, (3) 10 May, (4) 30 May.

The four 'infecter units' on each plot were sampled in the usual manner at the end of the season (23 September), and the total number of tubers taken from each plot was double that taken in previous seasons.

Aphids. 1949 differed from the earlier years because of a large spring migration of *Myzus persicae*, shown by trap records (Fig. 1). The maximum population of *M. persicae* on the plants was reached at the beginning of June, after which the population declined rapidly as a result of attacks by parasites. *Macrosiphum euphorbiae* was never numerous; *Aphis rhamni* reached a low maximum in early July.

Aphids were counted on 4-5 July in the same manner as in 1948, except that to estimate the size of the plants the total number of leaves was counted on four plants instead of two. The data for total aphids (mainly *A. rhamni*), and for *A. rhamni* alone were analysed statistically (Table 6). As in 1948, the earlier the potatoes were planted, the higher was the aphid population. Manurial treatments showed no significant effects, agreeing with the 1948 data for *A. rhamni*, but though non-significant, K reduced the number of aphids. After the first week of July the aphid population declined rapidly and remained less than one per leaf for the rest of the season.

Leaf roll and rugose mosaic. The mean incidence of disease in the September

TABLE 4. 1948 experiment (percentages transformed to degrees)

Incidence of leaf roll

		Plantings				Mean
		1	2	3	4	
Mean	(± 2.57)	33.0	30.0	32.9	31.6	31.9
No D	(± 3.63)	26.3	25.6	27.1	27.3	26.6
D		39.7	34.4	38.6	35.9	37.1
Response to D	(± 5.14)	13.4	8.8	11.5	8.6	10.5*
No N	(± 3.63)	27.3	32.9	30.6	29.1	30.0
N		38.7	27.1	35.1	34.1	33.7
Response to N	(± 5.14)	11.4	-5.8	4.5	5.0	3.7*
No P	(± 3.63)	35.0	29.0	30.9	26.9	30.5
P		31.0	31.0	34.8	36.2	33.3
Response to P	(± 5.14)	-4.0	2.0	3.9	9.3	2.8*
No K	(± 3.63)	37.3	29.2	30.6	29.5	31.6
K		28.7	30.8	35.1	33.7	32.1
Response to K	(± 5.14)	-8.6	1.6	4.5	4.2	0.5*

* S.E. ± 2.57 .Responses to manures (± 3.63)

Response to	D		N		P		K	
	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D	.	.	11.1	10.1	7.9	13.3	9.1	12.1
N	4.2	3.2	.	.	-2.2	9.6	0.7	6.7
P	0.1	5.5	-3.1	8.7	.	.	0.2	5.4
K	-1.1	1.9	-2.6	3.4	-2.2	3.0	.	.

Incidence of rugose mosaic

		Plantings				Mean
		1	2	3	4	
Mean	(± 2.71)	20.3	19.7	21.4	18.2	19.9
No D	(± 3.83)	17.0	16.7	16.2	11.4	15.3
D		23.7	22.7	26.7	25.0	24.5
Response to D	(± 5.42)	6.7	6.0	10.5	13.6	9.2*
No N	(± 3.83)	23.2	21.4	20.9	18.6	21.0
N		17.4	17.9	22.0	17.9	18.8
Response to N	(± 5.42)	-5.8	-3.5	1.1	-0.7	-2.2*
No P	(± 3.83)	24.6	17.0	19.3	18.0	19.7
P		16.0	22.4	23.6	18.4	20.1
Response to P	(± 5.42)	-8.6	5.4	4.3	0.4	0.4*
No K	(± 3.83)	17.3	20.0	20.2	17.3	18.7
K		23.4	19.4	22.6	19.2	21.1
Response to K	(± 5.42)	6.1	-0.6	2.4	1.9	2.4*

* S.E. ± 2.71 .Responses to manures (± 3.83)

Response to	D		N		P		K	
	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D	.	.	7.6	10.8	10.7	7.7	11.8	6.6
N	-3.8	-0.6	.	.	-6.4	2.0	-8.5	4.1
P	1.9	-1.1	-3.8	4.6	.	.	-3.3	4.1
K	5.0	-0.2	-3.9	8.7	-1.3	6.1	.	.

samples was 6.5 % leaf roll and 21 % rugose mosaic. The incidence of leaf roll decreased steadily with later planting, and the reduction between third and fourth plantings was significant. The incidence of rugose mosaic was significantly less in samples from the fourth planting than in the three earlier plantings (Table 7). Neither leaf roll nor rugose mosaic showed any significant response to any of the manurial treatments.

TABLE 5. 21-22 July, 1948. Log transformation ($\log_{10} N + 1$) of aphids per plant and mean responses to fertilizers

Treatments ...	Means				Mean responses				S.E.
	Plantings				Fertilizers				
	1	2	3	4	D	N	P	K	
Total aphids	3.34	3.31	3.01	2.56	0.15	0.07	0.19	-0.13	±0.055
Total alata	1.58	1.54	1.38	1.19	0.09	0.11	0.37	-0.11	±0.105
<i>M. persicae</i>	2.23	2.20	2.05	1.83	0.16	0.10	0.26	-0.08	±0.061
<i>M. euphorbiae</i>	2.89	2.73	2.40	1.71	0.17	0.10	0.27	-0.20	±0.066
<i>A. rhamni</i>	2.68	2.90	2.38	1.14	0.14	-0.15	0.13	-0.21	±0.118

TABLE 6. 4-5 July, 1949. Log transformation ($\log_{10} N + 1$) of aphids per plant and mean responses to fertilizers

	Means				Mean responses				S.E.
	Plantings				Fertilizers				
	1	2	3	4	D	N	P	K	
Total aphids	1.60	1.36	1.19	0.46	0.03	0.10	0.05	-0.11	± 0.084
<i>A. rhamni</i>	1.45	1.20	1.02	0.35	0.01	0.09	-0.03	-0.09	± 0.095

1950 EXPERIMENT

The design and treatments were the same as in 1949. Planting dates were:

(1) 31 March, (2) 21 April, (3) 12 May, (4) 2 June.

Tuber samples were taken at the end of the season on 25 September.

Aphids. As in 1949 there was a large spring migration (Fig. 1), but during 1950 coccinellids were exceptionally numerous and they kept the aphid population at a low level throughout the summer. Low maximum populations on the foliage were reached by *Myzus persicae* and *Aphis rhamni* in the third week in June, and by *Macrosiphum euphorbiae* in mid-July.

Leaf roll and rugose mosaic. The mean incidence of disease in the September samples was 4 % leaf roll and 3 % rugose mosaic. Both were significantly less in the two later plantings than in the two earlier (Table 8). Both diseases were increased by K, leaf roll particularly in the first planting and in the absence of D. Rugose mosaic was significantly increased by D in the first and fourth plantings, and by K in the second and third.

TABLE 7. 1949 experiment (percentages transformed to degrees)

Incidence of leaf roll

		Plantings				Mean
		1	2	3	4	
Mean	(± 2.09)	19.6	15.4	14.6	9.9	14.8
No D	(± 2.96)	19.9	17.1	12.9	8.0	14.4
D		19.2	13.8	16.3	11.7	15.2
Response to D	(± 4.18)	-0.7	-3.3	3.4	3.7	0.8*
No N	(± 2.96)	17.2	11.7	17.2	10.7	14.2
N		21.9	19.2	12.0	9.0	15.6
Response to N	(± 4.18)	4.7	7.5	-5.2	-1.7	1.4*
No P	(± 2.96)	22.8	16.8	12.5	8.1	15.0
P		16.3	14.1	16.7	11.6	14.7
Response to P	(± 4.18)	-6.5	-2.7	4.2	3.5	-0.3*
No K	(± 2.96)	21.3	14.0	14.1	10.0	14.8
K		17.8	16.8	15.1	9.7	14.8
Response to K	(± 4.18)	-3.5	2.8	1.0	-0.3	0*

* S.E. ± 2.09 .Responses to manures (± 2.96)

Response to	D		N		P		K	
	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D	.	.	-0.5	2.1	0.4	1.2	4.0	-2.4
N	0	2.6	.	.	-0.6	3.2	0.6	2.0
P	-0.8	0	-2.3	1.5	.	.	1.6	-2.4
K	3.2	-3.2	-0.7	0.7	2.0	-2.0	.	.

Incidence of rugose mosaic

		Plantings				Mean
		1	2	3	4	
Mean	(± 3.28)	28.9	30.5	27.8	21.8	27.3
No D	(± 4.64)	26.1	29.2	22.7	21.4	24.8
D		31.7	31.8	32.8	22.3	29.7
Response to D	(± 6.57)	5.6	2.6	10.1	0.9	4.9*
No N	(± 4.64)	27.8	29.5	25.8	22.3	26.3
N		30.0	31.6	29.6	21.3	28.1
Response to N	(± 6.57)	2.2	2.1	3.8	-1.0	1.8*
No P	(± 4.64)	30.1	32.6	27.8	24.3	28.7
P		27.7	28.5	27.6	19.4	25.8
Response to P	(± 6.57)	-2.4	-4.1	-0.2	-4.9	-2.9*
No K	(± 4.64)	26.2	27.4	28.2	21.0	25.7
K		31.6	33.7	27.3	22.6	28.8
Response to K	(± 6.57)	5.4	6.3	-0.9	1.6	3.1*

* S.E. ± 3.28 Responses to manures (± 4.64)

Response to	D		N		P		K	
	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D	.	.	1.6	8.0	4.9	4.7	5.0	4.6
N	-1.4	5.0	.	.	-1.1	4.7	7.5	-3.9
P	-2.8	-3.0	-5.8	0	.	.	-1.6	-4.2
K	3.3	2.9	8.8	-2.6	4.4	1.8	.	.

TABLE 8. 1950 experiment (percentages transformed to degrees)

		Incidence of leaf roll				
		Plantings				
		1	2	3	4	Mean
Mean	(± 2.01)	15.3	16.1	4.7	9.0	11.3
No D	(± 2.84)	11.6	16.3	3.6	7.8	9.8
D		19.0	15.9	5.8	10.3	12.8
Response to D	(± 4.02)	7.4	-0.4	2.2	2.5	3.0*
No N	(± 2.84)	16.2	14.1	2.8	9.2	10.6
N		14.4	18.1	6.7	8.9	12.0
Response to N	(± 4.02)	-1.8	4.0	3.9	-0.3	1.4*
No P	(± 2.84)	15.7	11.9	4.7	9.5	10.5
P		14.9	20.2	4.7	8.6	12.1
Response to P	(± 4.02)	-0.8	8.3	0.0	0.9	1.6*
No K	(± 2.84)	10.4	15.2	4.2	7.9	9.4
K		20.2	17.0	5.2	10.2	13.1
Response to K	(± 4.02)	9.8	1.8	1.0	2.3	3.7*

* S.E. ± 2.01 .

		Responses to manures (± 2.84)							
		D		N		P		K	
Response to		Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D		.	.	1.6	4.2	4.5	1.3	7.1	-1.3
N		0.2	2.8	.	.	2.1	0.9	4.2	-1.2
P		3.2	0.0	2.2	1.0	.	.	0.6	2.6
K		7.9	-0.5	6.4	1.0	2.7	4.7	.	.

Incidence of rugose mosaic						
		Plantings				
		1	2	3	4	Mean
Mean	(± 1.49)	14.3	12.2	8.4	5.8	10.2
No D	(± 2.11)	8.8	10.7	6.6	1.1	6.8
D		19.7	13.7	10.2	10.4	13.5
Response to D	(± 2.98)	10.9	3.0	3.6	9.3	6.7*
No N	(± 2.11)	13.0	12.3	6.3	3.6	8.8
N		15.5	12.1	10.5	7.9	11.5
Response to N	(± 2.98)	2.5	-0.2	4.2	4.3	2.7*
No P	(± 2.11)	13.2	11.4	6.6	5.6	9.2
P		15.3	12.9	10.2	5.9	11.1
Response to P	(± 2.98)	2.1	1.5	3.6	0.3	1.9*
No K	(± 2.11)	11.5	8.2	5.1	5.2	7.5
K		17.0	16.2	11.7	6.3	12.8
Response to K	(± 2.98)	5.5	8.0	6.6	1.1	5.3*

* S.E. ± 1.49 .

		Responses to manures (± 2.11)							
		D		N		P		K	
Response to		Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.
D		.	.	5.9	7.5	6.8	6.6	7.7	5.7
N		1.9	3.5	.	.	2.9	2.5	0.7	4.7
P		2.0	1.8	2.1	1.7	.	.	0	3.8
K		6.3	4.3	3.3	7.3	3.4	7.2	.	.

DISCUSSION

The experiments were made with Scotch Majestic potatoes 'Stock Seed' grade (certified as originally less than 0.02 % leaf roll and rugose mosaic), artificially contaminated by adding approximately 0.5 % leaf roll and 0.5 % rugose mosaic 'infectors' in known positions. The records of incidence of leaf roll and rugose mosaic refer only to the five plants on each side of an 'infector' plant.

The results are interpreted as evidence that date of planting affects the incidence of leaf roll and rugose mosaic in two ways. Its influence on date of emergence of the shoots above ground determines the presence or absence of plants during the spring migration of aphids. It also determines the maturity and susceptibility of the plants during the summer migration of aphids.

In 1949 and 1950, when the spring migrations were large and the summer migrations small, most of the infections probably occurred in May and June when the later plantings had not emerged, and there was less disease in the late than in the early planted crops. In 1948, on the other hand, when a very small spring migration was followed by a summer migration which lasted from mid-June to early August, there were no differences in the incidence of the diseases in the different plantings. 1947 began, like 1948, with very few aphids, but many *Myzus persicae* colonized the crop on 16 July. Some of these were evidently carrying leaf-roll virus and they infected many plants, with no apparent spatial relation to the planted leaf-roll 'infectors'. Presumably at this time the younger plants were more susceptible than the older, as the average incidence of leaf roll was 58 % in the plots planted early and 82 % in those planted later. In 1945 also there was a very small spring migration, and the summer migrants appear to have spread most of the leaf roll, with the result that the later plantings had a higher incidence of leaf roll than the early.

The incidence of rugose mosaic equalled or exceeded leaf roll in 1949 and 1950, the only years with large spring migrations of *M. persicae* followed by small summer migrations. In the other four years with summer migrations greater than the spring, leaf roll considerably exceeded rugose mosaic.

The different manurial treatments had widely different results. Experiments with four plantings in each of five years, and with two in 1947, gave a total of twenty-two manurial trials. In general, D, N, and K increased the incidence of leaf roll and rugose mosaic, sometimes significantly, whereas P had no obvious effect (Table 9). The mean yields, disease incidence and manurial effects of the four experiments of the same design (1946, 1948, 1949, 1950) are shown in Table 10. The lack of response to P is common in field experiments at Rothamsted where most of the soil has adequate phosphate.

Although, as was expected, variations in date of planting and of manuring potato crops do affect the incidence of virus diseases, their effects are fortunately small—fortunately, because such variations also greatly affect the yield of tubers

and most of the variations that increase yield also increase the incidence of virus diseases. Despite the correlation between yield and virus disease, there is no doubt that the grower of ware potatoes should aim at the maximum yield of tubers. The financial loss from increased incidence of disease caused by early planting and adequate manuring will be insignificant compared with the gain from increased yield. An increase in leaf roll and rugose mosaic reduces the value of seed-size tubers for subsequent planting, but causes inappreciable loss of yield in the year of infection. It is the practice in most parts of England for ware-growers to discard their stock after one or two seasons, and it is not likely that this practice will be affected by early planting and generous manuring for maximum yield.

TABLE 9. *Proportion of trials in which manurial treatments increased disease*

(Significant increases in brackets)

Manure	Total no. of trials	No. of trials with increased	
		Leaf roll	Rugose mosaic
D	22	17 (5)	20 (6)
N	22	17 (3)	12 (0)
P	16	7 (1)	9 (1 sig. decrease)
K	18	10 (1)	13 (3)

TABLE 10. *Means of four years' experiments with the same design*

Planting date	Yield tons/acre	Leaf roll %	Rugose mosaic %
4 April	10.31	17.9	10.4
24 April	8.97	11.3	11.9
12 May	8.85	11.2	9.5
30 May	7.50	10.5	6.4

Mean effects

	Increase in yield tons/acre	Increase in percentage	
		Leaf roll	Rugose mosaic
D	2.62	5.7	5.8
N	1.11	2.0	0.4
P	0.61	0.2	-0.8
K	1.17	0.4	2.7

These experiments were made in a ware-growing area, and are not necessarily applicable to seed areas, where crops are usually planted later. The seed-growing areas are mostly distant from places where aphids overwinter, and migrants do not colonize the crops until July. These migrants might carry leaf-roll virus, as did those which infested the Rothamsted crops in 1947, and the younger the crops, the more susceptible to infection they will be. Planting early in seed areas, therefore, might reduce the incidence of virus diseases, but the effects of varying dates of planting and manuring on the health of seed stocks need testing in seed-growing areas before any recommendations can be made.

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THE CHANGING NUMBERS OF *APHIS FABAE* SCOP., FLYING AT CROP LEVEL, IN RELATION TO CURRENT WEATHER AND TO THE POPULATION ON THE CROP

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(With 7 Text-figures)

Aphis fabae Scop. flying over bean crops in the summer usually show a double peak of aerial density during the day; scarcely any flight occurs at night. The first peak is thought to be composed mainly of alatae moulted since the previous evening and the decline of the peak to their depletion as they fly away from the crop. The second peak is probably composed mainly of alatae moulted during the same day. Lack of flight at night is due partly to low temperature, partly to lack of alatae old enough to fly and probably also to low light intensity.

Contrary to expectation changes in aerial density from hour to hour are only very weakly correlated with weather factors, especially wind-speed. The total numbers in each of the two peaks do show a low but significant correlation with both wind-speed and temperature in one case, but even then only a relatively small amount of the variation in aerial numbers is associated with weather changes. Other factors, particularly rapid changes in the numbers of alatae on the crop caused by moulting, accumulation before flight and depletion by flight evidently obscure changes due simply to varying flight behaviour. Thus because of large populations on the crop, it is possible to have quite large numbers in the air even when the weather is relatively unfavourable for flight.

In addition to these observations, the relation between total numbers caught at different wind-speeds shows that most of the migration occurred in winds when the aphids could have had no control over the general direction of flight. The current view that most migration takes place only in calm weather can therefore no longer be held.

INTRODUCTION

The flight behaviour of individual aphids is strongly affected by meteorological factors; resting aphids will not take flight if exposed to even a slight breeze. The sensitivity of these reactions have encouraged the view that change in the numbers of aphids flying from day to day is largely a matter of weather-induced behaviour, and this has been tacitly acknowledged by attempts to correlate changes in aerial numbers with current weather conditions. Moreover, field work seemed to support the plausible idea that most migration occurs in calm weather.

But although changes in numbers flying from month to month are recognized as population fluctuations, there has been no critical assessment of the part played by population changes in the more rapid variation in aerial numbers usually ascribed to weather and behaviour. The present work aimed to do this. Two disk-dropping suction traps, one sheltered, one exposed, and near infested beans,

trapped flying alienicolae of *Aphis fabae*, hour by hour, day and night over the whole infestation period of 5-6 weeks; simultaneously hourly meteorological records were taken. It was thought that differences in catch from hour to hour would be due to behaviour changes, that these could be related to the weather and also compared with the changing level of population by statistical methods.

The results were unexpected: numbers flying and their changes were only weakly correlated with the weather; most migration occurred in winds which would prevent aphids from controlling their flight direction; the statistical procedure for distinguishing behaviour and population changes was inappropriate and unreliable. In describing these results this paper leaves many problems unsolved; but it attempts to place behaviour and population factors in aphid migration more in perspective and to show the pattern of the problem in more detail than hitherto.

METHODS

The traps

Two identical 9 in. flared suction traps, A and B, were used, each sampling approximately 20,000 cu.ft. of air/hr. in still air. When a flare was fitted to the mouth of the trap, measurements showed that the adverse effect of wind on the efficiency of the trap was eliminated for winds up to 12 m.p.h. (Johnson, 1950). However, it has been found recently that a correction for wind-speed is still necessary.

TABLE 1. *Correction factor for an exposed 9 in. Vent-axia suction trap, by which the volumetric air flow may be multiplied to give a constant value when the trap operates in various wind-speeds*

Wind-speed (m.p.h.)	Factor	Wind-speed (m.p.h.)	Factor
0	1.00	11	1.23
1	1.01	12	1.30
2	1.02	13	1.38
3	1.02	14	1.49
4	1.03	15	1.63
5	1.04	16	1.81
6	1.06	17	2.05
7	1.08	18	2.40
8	1.11	19	2.89
9	1.13	20	3.71
10	1.18	21	6.29

Trap B encountered much wind, and the numbers of aphids caught have been corrected to values proportional to the volume of air the trap would have sampled had it operated as efficiently in windy as in calm weather. The mean wind-speed for each hour at 40 ft. (at the meteorological enclosure, $\frac{1}{4}$ mile away) was used for this correction; to have converted this to a value at trap level would have given only a doubtful increase in accuracy. The correction factor (Table 1) has not been applied to trap A which was sheltered.

In correcting, the catch is assumed to be proportional to the volume of air sampled. Any error due to a varying proportion of aphids being carried beyond the trap by strong variable winds is likely to be small compared to other errors and would only enhance the conclusions in this paper.

Trap positions

Trap A was in the centre of a small plot 94×30 ft. planted in the spring with sixty-four rows of Garton's Yearling field beans, at 4 beans/ft., 18 in. between rows. At maturity the plot contained approximately 8800 stems. No stems were allowed to touch the trap, whose opening was kept 1–2 in. above the top of the crop. The situation was a sheltered garden.

Trap B was in the middle of a potato plot, 153×93 ft., behind the Rothamsted Laboratories. The mouth of the trap was about 2 ft. above the top of the crop; 54 ft. to the north was a thick border of flowering sugar beet and 60 ft. to the southwest a plot of field beans, 72×24 ft., both heavily infested with *Aphis fabae*. The situation was exposed.

Meteorological records

Trap A. A calibrated thermograph and maximum and minimum thermometers were kept in a Stevenson screen 2 ft. above the ground and at the edge of the crop a few feet from the trap. A continuous recording sensitive cup anemometer stood level with the rim of the trap, 8 ft. away.

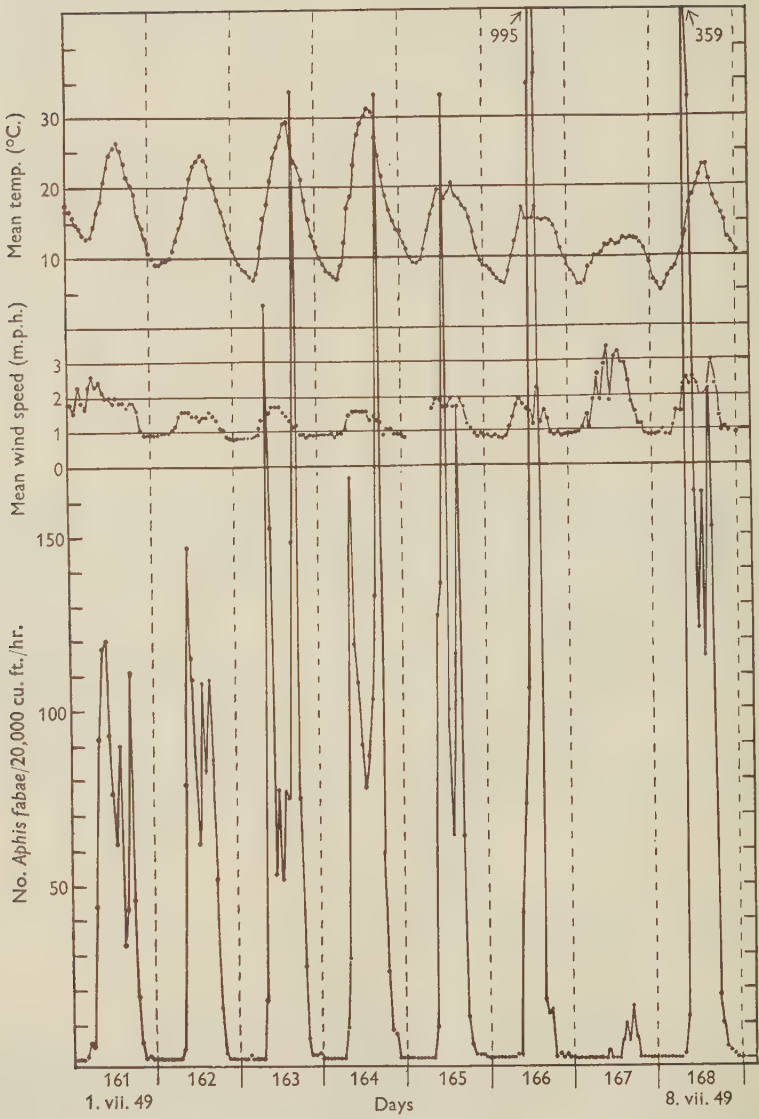
Trap B. No instruments were available at the trap site, but $\frac{1}{4}$ mile to the southwest, temperatures and relative humidities were recorded with a thermo-hygrograph in a Stevenson screen at 5 ft., and a three-cup anemometer at 40 ft. recorded the hourly run of wind. Hours of sunshine and the rainfall were measured with standard instruments. The temperatures recorded in the screens are not necessarily the same as those in different parts of the crops. While recognizing the importance of microclimate in aphid behaviour population factors outweigh it here.

CHANGES IN NUMBERS OF APHIDS FLYING IN RELATION TO TEMPERATURE AND WIND-SPEED

Temperature and wind-speed are perhaps the two major meteorological factors affecting flight and they have been considered together.

Density change during the day near the crop

Aphids begin to fly soon after sunrise, and their density in the air near the crop reaches a maximum usually before noon; it then declines, but builds up again in the afternoon or evening to a second peak, after which it subsides; scarcely any flight occurs at night. Although these two peaks, either of which may be the actual daily maximum, do not occur every day (sometimes a single large peak occurs, sometimes several smaller ones), they are common with most species of aphids. Examples may be seen in Figs. 1 and 2 for *A. fabae*; other species have been cited by Eastop (1951).



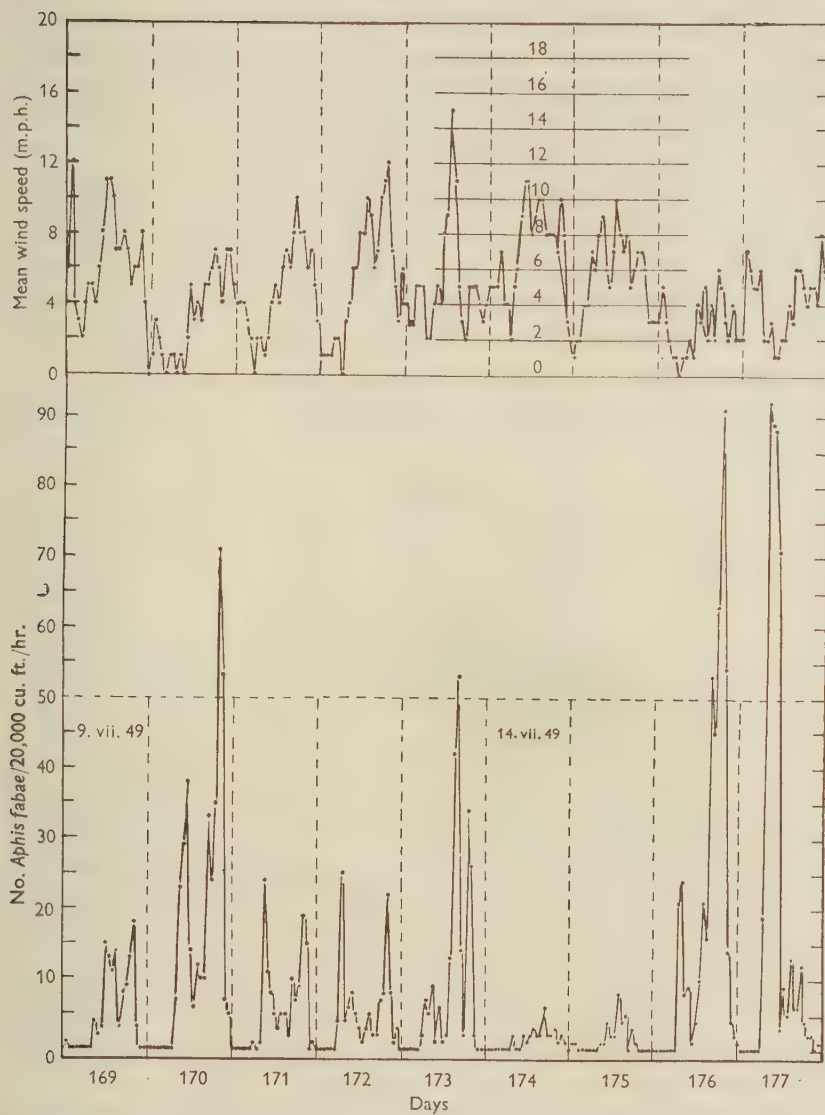


Fig. 2. *A. fabae*, Trap B. Numbers caught and mean wind-speed per hour (at 40 ft.) for 9 consecutive days.

At first sight these changes appear to be fairly simply related to wind-speed and temperature; for density increases with a rise of temperature in the morning, and drops as temperature falls in the evening. The decline in density between the two daily maxima is not, however, a simple effect on flight activity of temperature or wind, for with trap A the peak densities and the subsequent declines occur at mean hourly temperatures as different as 15.0 and 31.9° C. Nor is the cessation of flight in the evening simply due to temperature falling below a threshold value; for the beginning and end of activity occur at screen temperatures as different as 7.4–8.0° and 17.5–19.6° C. respectively. Moreover, the start occurred at such widely different temperatures as 7.4–8.0° C. (day 170) and 16.6–19.6° C. (day 160) (Table 2). Corresponding microclimatic conditions in the crop also would have shown great differences, although as Muller & Unger have shown (1951) the

TABLE 2. *Time (B.S.T.), maximum, minimum temperature and mean wind-speed of the hour of start and finish of flight activity on some consecutive days with Trap A*

Day	Hour	Start (° C.)	Mean wind-speed (m.p.h.)	Hour	Finish (° C.)	Mean wind-speed (m.p.h.)
160	9–	16.6–19.6	1.3	23–	17.1–17.5	1.6
161	4–	13.8–14.9	1.8	22–	13.1–15.0	0.8
162	8–	13.2–15.0	1.4	20–	17.5–19.6	1.1
163	8–	12.2–16.6	1.3	23–	11.6–13.5	0.8
164	7–	8.7–13.0	0.9	21–	14.1–14.7	1.0
165	—	—	—	22–	9.5–11.4	0.8
166	8–	10.2–12.7	1.4	18–	15.8–16.3	0.8
167	10–	12.5–13.9	2.9	23–	7.0–8.0	0.8
168	8–	10.2–11.5	1.6	22–	12.2–13.0	1.1
169	8–	12.7–15.2	1.4	21–	11.2–13.5	0.8
170	6–	7.4–8.0	0.8	23–	12.1–13.5	0.8

temperature on the upper surface of the leaf from which the aphids take off is about 4° C. higher than the temperature in the air a few centimetres away. Differences in temperature relations observed here may be due to the lack of power of the trap to sample from low early morning densities. It is likely, however, that when catches ceased at a high temperature there were no alatae ready to fly or possibly flighting was inhibited by low flight intensity.

Again, there is no obvious relation with wind-speed; for the first and second peaks may occur when flight conditions are unfavourable because of relatively high winds; while the minimum density between the peaks may coincide with optimal conditions for take-off, namely, low wind and high temperature.

For example, on day 160 (trap B, Table 3) the density rises between noon and 19.00 hr., while the wind is also rising from complete calm to an appreciable breeze with a mean hourly value of 7 m.p.h.; thereafter density falls with a *falling* wind-speed.

Similarly, day 168 (trap B, Table 3) was a day of high winds; yet in spite of them density increased to its maximum at 16.00 hr. Day 170 was not calm; but the general

tendency was for density to increase while the wind also increased in speed. The double daily peaks are more consistent in sheltered trap A than with exposed trap B, where suppression of peaks is more in evidence (Fig. 2).

TABLE 3. *Corrected hourly densities (D), in relation to mean hourly wind-speed (m.p.h.) for trap B*

Hr. (B.S.T.)	Day 160		Day 168		Day 170	
	m.p.h.	D	m.p.h.	D	m.p.h.	D
7-	—	—	—	—	0	0
8-	3	0	—	—	1	7.1
9-	3	5.1	17	0	0	23.0
10-	3	13.3	18	14.4	2	29.6
11-	0	10.0	16	7.2	5	39.5
12-	2	5.1	19	26.0	3	14.3
13-	5	11.4	13	13.8	4	6.2
14-	3	11.2	9	15.8	3	12.2
15-	0	10.0	13	34.5	5	10.4
16-	0	17.0	13	34.5	5	10.4
17-	5	26.0	9	29.4	6	35.0
18-	6	23.3	8	12.2	7	25.9
19-	7	76.7	9	7.9	6	37.1
20-	5	21.8	12	7.8	4	73.1
21-	4	8.2	16	1.8	7	57.2
22-	4	0	15	0	7	7.6
23-	—	—	—	—	5	5.2

TABLE 4. *Multiple regressions for differences of successive catches of log (density/hour + 1) on corresponding differences of mean hourly temperatures and on wind-speed throughout the infestation period*

Trap A. Temperature in ° C. Wind-speed (m.p.h.) is the mean for the two successive hours. Only density rises considered.

Trap B. Temperature in ° F. Wind-speed (m.p.h.) is the mean for the later hour.

b = partial regression coefficients for log (density/hour + 1) on x and z .

x = temperature differences.

z = wind-speed.

ss = sum of squares for regressions as % total sum of squares.

n = degrees of freedom.

	b	S.E.	$P=0.05$	ss	n
Trap A					
x	0.0796	0.0161	Sig.		
z	-0.1242	0.0672	Not sig.	20 %	111
Trap B					
x	0.0120	0.0065	Not sig.		
z	0.0011	0.0013	Not sig.	1.4 %	261

The rate of density change from hour to hour

With trap A rate of density change from hour to hour shows a significant positive regression on temperature change, but the regression with wind-speed though negative is not statistically significant. With trap B neither regressions, on temperature differences nor on wind-speed, were statistically significant (Table 4).

The total variation in rate of density change from hour to hour accounted for by the two factors, wind-speed and temperature, is small (20 and 1.4 % of sum of squares in traps A and B respectively).

These results are from the partial regressions for differences of consecutive values of $\log(\text{hourly catch} + 1)$ on corresponding differences in temperature and on wind-speed. Wind-speed was expressed as the mean wind-speed for the two successive hours (trap A) or the mean wind-speed for the later hour (trap B); differences in wind-speed from hour to hour were also used but with no different result. It was thought advisable to eliminate, as much as possible, any effects of depletion of the population on density change, and so only rises in density were at first analysed. Subsequent inclusion of the falling density values gave no different results as Fig. 3 shows graphically.

The lack of obvious effects of wind on density could be explained thus: the two diurnal peaks are due to different batches of accumulated alatae (see p. 544) whose depletion by flight could produce a diminishing catch with a rising percentage take-off. Thus if from 100 70 % fly, 30 will remain and even 100 % take-off will only cause 30 to fly.

Fluctuations in successive 'half-day' periods

The 'half-day' period and method of expressing data

The effect of meteorological factors on the number of aphids flying was considered also with total daily catches and with *mean* hourly catches for the day, but these periods apparently were too coarse. It was considered better biologically to divide the daily flight period into two parts, the division coming at the trough after the first peak of density and tentatively to regard each part, or 'half-day', as encompassing a distinct phase.

There is reason to believe that the two peaks of density during the day reflect two peaks of alate population. The hourly densities of which these are made depend not only on take-off rates but also on rates of depletion of available alatae; the latter may mask the effect of weather on take-off and thus lead to the lack of correlation shown in the previous pages. With the total number of aphids caught during a 'half-day', however, depletion effects within the 'half-day' need not be considered and a correlation of numbers with weather might be expected.

It was usually easy to determine the time for dividing a day into two such parts, but on a few days when the double peak did not show up at all, or was confused by a number of smaller peaks, a somewhat arbitrary point was taken. These 'half-day' periods are thus not necessarily of equal length, varying from about 4 to 16 hr. (Tables 5 and 6).

It should be clearly understood that here we are not dealing with density (which is numbers per unit volume of air per unit time), but with the numbers flying within a biologically significant, but variable, time period. This period varies

because of the production and behaviour of the insects, not because the traps were operated for variable times, for these ran continuously.

Aphid numbers were expressed as the log (total numbers caught per 'half-day'), which for convenience is called $\log \text{total}/\frac{1}{2}\text{-D}$. The log (mean number caught/hr.) proved less profitable an expression than the more straightforward $\log \text{total}/\frac{1}{2}\text{-D}$.

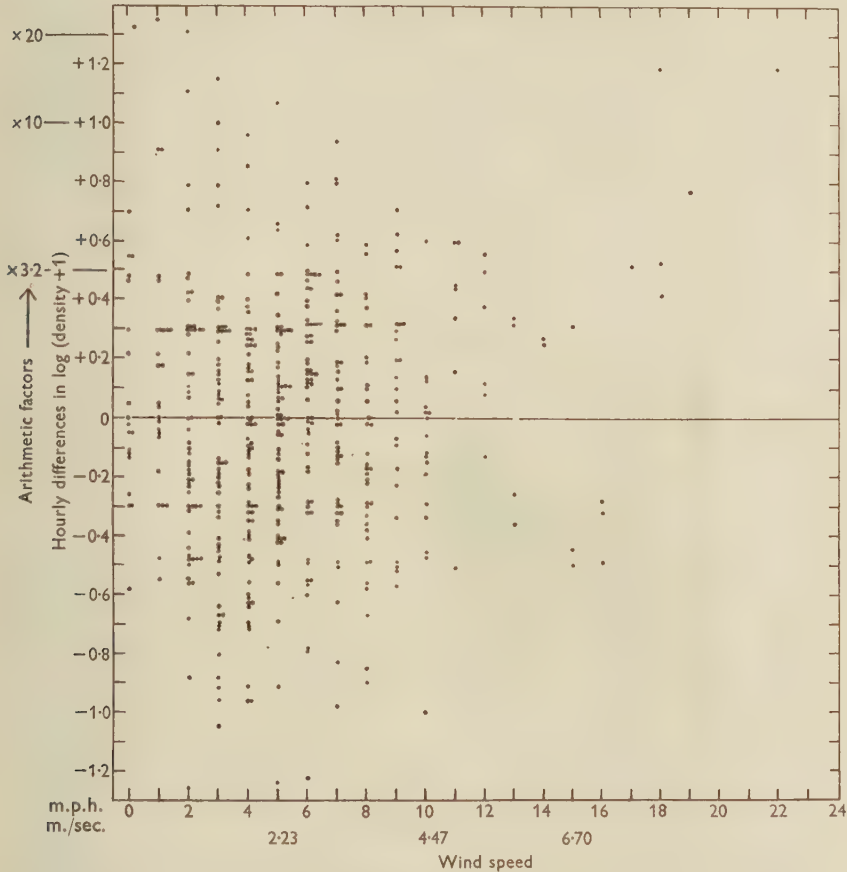


Fig. 3. Trap B. Differences of $\log (\text{density} + 1)$ between consecutive hours in relation to hourly wind-speed.

Temperature and wind-speed were expressed as the mean temperature and mean wind-speed for each 'half-day' catch period—that is, the sum of the mean temperature (or wind-speed) of each hour when aphids were caught, divided by the number of such hours in each half-day. A running mean of $\log \text{total}/\frac{1}{2}\text{-D}$ for three 'half-day' periods has been used and this is expressed as ' $3 \frac{1}{2}\text{-DRM}$ '.

TABLE 5. *Basic data for Aphis fabae from trap A, 1949, in 'half-day' periods from 23 June (Day 153)*

Day	Catch period (hr.)	No. caught	Mean temp. (° C.)	Mean wind-speed (m.p.h.)	Day	Catch period (hr.)	No. caught	Mean temp. (° C.)	Mean wind-speed (m.p.h.)
153	5	14	25·3	3·41	171	10	1257	22·6	1·45
	5	8	19·2	2·67		7	712	21·2	1·08
154	6	27	20·2	—	172	10	1899	24·2	1·42
	5	19	22·5	—		8	568	25·6	1·34
155	7	45	24·3	1·15	173	9	664	19·8	1·28
	7	40	21·8	0·89		11	1011	21·5	1·03
156	7	65	20·8	1·04	174	7	456	20·8	1·53
	5	90	28·0	1·26		10	586	23·4	1·31
157	3	91	19·4	—	175	6	795	19·8	1·50
	8	136	26·3	1·05		6	262	21·3	1·36
158	9	208	25·0	1·88	176	7	495	16·9	0·93
	6	126	25·5	1·05		8	542	17·2	0·92
159	11	324	20·5	1·89	177	12	513	16·5	0·98
	7	120	20·6	1·78		4	31	14·9	1·00
160	5	191	17·7	1·11	178	8	139	12·5	2·38
	11	401	26·0	1·13		5	72	15·7	1·58
161	12	728	20·6	2·08	179	6	85	14·7	1·05
	6	229	21·3	1·60		7	87	18·6	1·27
162	6	505	19·9	1·50	180	6	52	18·7	1·09
	7	454	23·5	1·36		6	33	20·1	0·96
163	7	573	20·3	1·44	181	6	24	21·4	1·29
	10	689	23·5	1·08		5	17	26·4	1·23
164	7	600	22·8	1·38	182	6	14	25·8	1·10
	8	698	26·3	1·23		7	15	25·5	1·01
165	6	712	19·7	1·88	183	5	13	22·8	1·00
	8	386	16·7	1·40		7	12	26·1	1·35
166	7	1498	15·6	1·65	184	6	9	24·7	1·76
	5	327	15·6	1·43		6	13	27·6	1·04
167	4	21	13·0	2·75	185	5	8	22·3	1·07
	3	22	12·5	1·36		2	5	30·6	1·19
168	9	1212	17·7	2·03	186	4	6	29·5	1·25
	7	378	17·8	1·83		2	5	27·9	1·02
169	8	1721	16·0	1·50	187	2	2	25·5	1·70
	7	535	17·1	1·21					
170	9	1185	17·1	1·24					
	10	1065	22·5	1·23					

TABLE 6. *Basic data for Aphis fabae from trap B, 1949, in 'half-day' periods from 23 June (Day 153)*

Day	Catch period (hr.)	No. caught	Corrected* catch	Mean temp. (° F.)	Mean wind-speed (m.p.h.)
153	8	—	—	65·7	—
154	6	13	13·8	62·0	4·50
	4	7	7·2	66·1	4·50
155	4	11	11·6	63·7	4·50
	10	111	113·4	72·6	3·50
156	6	71	72·5	67·2	3·00
	10	96	97·3	74·5	4·00
157	16	57	59·2	68·2	4·50
	7	66	70·1	79·1	5·00
158	11	34	35·2	72·2	4·25
	8	79	82·1	76·5	3·75

* Corrected on hourly catches.

TABLE 6 (continued)

Day	Catch period (hr.)	No. caught	Corrected catch*	Mean temp. (° F.)	Mean wind-speed (m.p.h.)
159	8	19	19.5	65.3	2.88
	9	97	104.0	70.5	6.22
160	6	35	35.5	64.1	2.33
	10	197	206.6	77.4	4.00
161	10	76	75.9	67.0	1.00
	8	74	79.7	69.6	6.25
162	6	58	59.1	68.6	2.67
	8	65	67.7	68.5	4.63
163	8	59	59.3	68.3	4.00
	9	161	167.9	74.3	3.89
164	10	52	53.6	66.1	4.50
	7	46	57.2	75.3	9.29
165	8	18	20.3	61.0	10.25
	5	78	81.9	66.1	4.00
166	8	53	72.6	58.2	9.30
	5	61	75.4	60.5	9.60
167	1	1	1.1	50.5	9.00
	1	1	2.4	52.8	18.00
168	5	43	77.2	60.6	15.00
	7	101	128.1	67.8	11.43
169	9	66	74.2	60.2	8.89
	5	51	54.2	65.4	6.40
170	6	117	119.7	64.8	2.50
	10	260	274.1	74.5	5.50
171	10	65	67.4	69.2	4.50
	6	61	66.9	76.0	7.00
172	8	56	58.6	75.2	5.63
	10	61	68.6	78.6	7.60
173	7	31	32.5	71.6	4.86
	6	126	133.5	75.7	6.83
	3	62	64.5	70.2	4.67
174	6	12	13.9	73.5	9.00
	8	19	20.9	71.9	6.75
175	7	12	13.0	66.8	5.71
	5	21	22.5	72.3	6.80
176	5	63	63.7	64.3	1.80
	12	377	388.1	63.7	3.33
177	7	363	367.5	61.9	1.86
	10	60	62.7	59.9	4.80
178	8	35	38.9	57.4	8.10
	4	22	23.1	60.8	5.00
179	4	9	11.7	57.6	10.00
	8	52	59.0	65.1	7.50
180	8	52	54.2	64.9	4.88
	6	52	53.4	67.0	3.33
181	3	11	11.5	64.5	6.00
	8	65	68.1	75.6	5.50
182	5	28	30.9	74.3	3.80
	9	56	57.5	77.8	3.78
183	7	26	30.1	69.9	4.57
	10	111	117.1	78.4	5.30
184	11	127	131.9	71.2	4.18
	6	33	34.0	79.8	3.83
185	7	40	40.8	69.5	2.57
	6	13	13.6	84.2	5.50
186	7	26	27.9	73.8	6.14
	7	42	45.2	80.4	5.86
187	1	1	1.0	65.0	3.00
	2	2	2.5	79.0	10.00
188	2	2	2.5	67.3	11.00
	3	7	7.8	74.5	8.33
189	1	1	1.3	70.0	8.50

* Corrected on hourly catches

Sequence of numbers trapped

The sequence of numbers trapped per half-day in traps A and B is shown, on an arithmetical scale, in Fig. 4. Basic data are given in Tables 5 and 6. In trap A a smooth but sharp increase culminates on 6 July, when, with a single peaked daily curve, a maximum of 995 *A. fabae* were caught in a single hour (Fig. 1); the curve declines to zero on about 30 July. This is obviously a population curve and closely follows a graph for the apterous population which was made at the same time.

Catches were higher with trap A than with trap B, no doubt partly due to its closer proximity to the infected stems, and at first sight the peak on 6 July seems to stand out as being due to a sudden rate of increase. This is not so, however, for the graph on a logarithmic scale (Fig. 4) shows that the rate of increase was fairly steady, and with no exceptional change on 5 or 6 July (days 165 and 166).

At trap A, the rapidity and magnitude of the population growth completely obscures the relatively small variations due to flight behaviour, so that there are no statistically significant regressions of density or its change with any of the weather factors considered here. Also, though calm, great hourly changes occurred.

With the data from trap B, however, relationships with current meteorological factors show up to a greater extent, both when the 'half-day' totals and their running means are used. These will now be considered.

Possible optimum conditions and effects from the previous half-day

Log total/ $\frac{1}{2}$ -D plotted against mean temperature, either with primary data or running means, shows an optimum condition associated with temperatures near 65° F. above which the catch diminishes (Fig. 5). There is no reason for regarding this as an optimum for flight activity for neither Broadbent's (1949) laboratory data nor hourly suction trap catches show such a condition, although with the latter population changes could obscure an optimum effect.

There may, however, be an optimum for moulting; for log total/ $\frac{1}{2}$ -D plotted on temperatures of the *previous* half-day also show an optimum, and observations show that the alatae caught could have moulted within the previous half-day or within a period close to it. Thus in parts of the graph in Fig. 6, e.g. days 166-170, the 3 $\frac{1}{2}$ -DRM of log total/ $\frac{1}{2}$ -D is positively correlated with mean temperature corresponding to the previous half-day; elsewhere it is not (days 155-165). But if each temperature above 68° is converted to an equivalent value below it (69 $\equiv$ 67°, 70 $\equiv$ 66°, etc.) then graphs for log total/ $\frac{1}{2}$ -D and converted temperature run closely together over much of the infestation period (Fig. 6).

There is some reason, too, for expecting an optimum; for Davidson (1925), Ewing (1916) and Rivnay (1938) showed that development of *A. fabae*, *A. avenae* and *Toxoptera aurantii* was optimal at 70, 65 and 72-77° F. respectively, although higher temperatures were not so depressive in effect as appears with the present data. The optimum for *M. persicae* is approximately 24° C. (Fenjves, 1945).

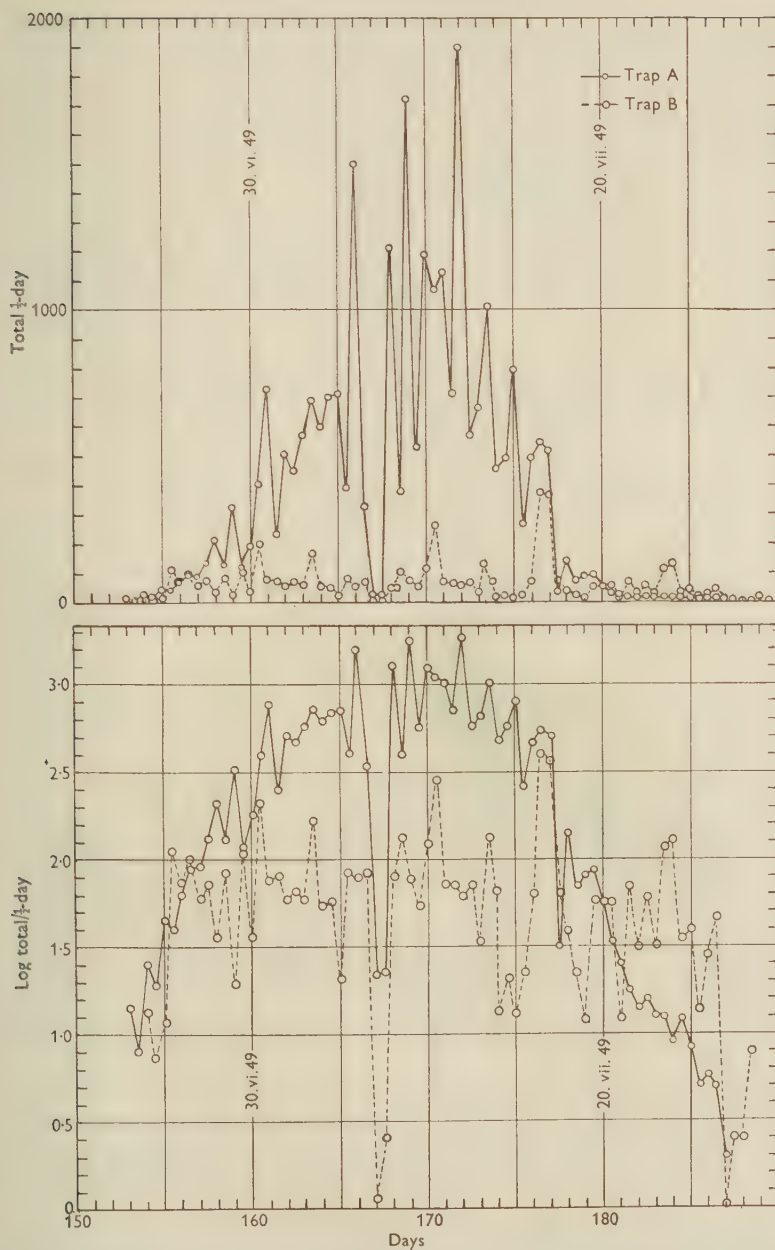


Fig. 4. Traps A and B. Total/ $\frac{1}{2}$ -day and log total/ $\frac{1}{2}$ -day throughout most of the infestation period

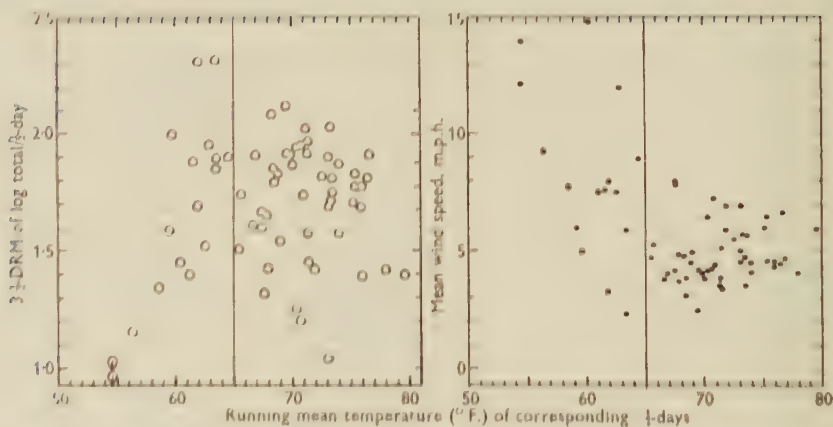


Fig. 5. Trap B. The relations of the 3 half-day running means of log total $\frac{1}{2}$ -day and mean wind-speed to mean temperature of previous $\frac{1}{2}$ -day.

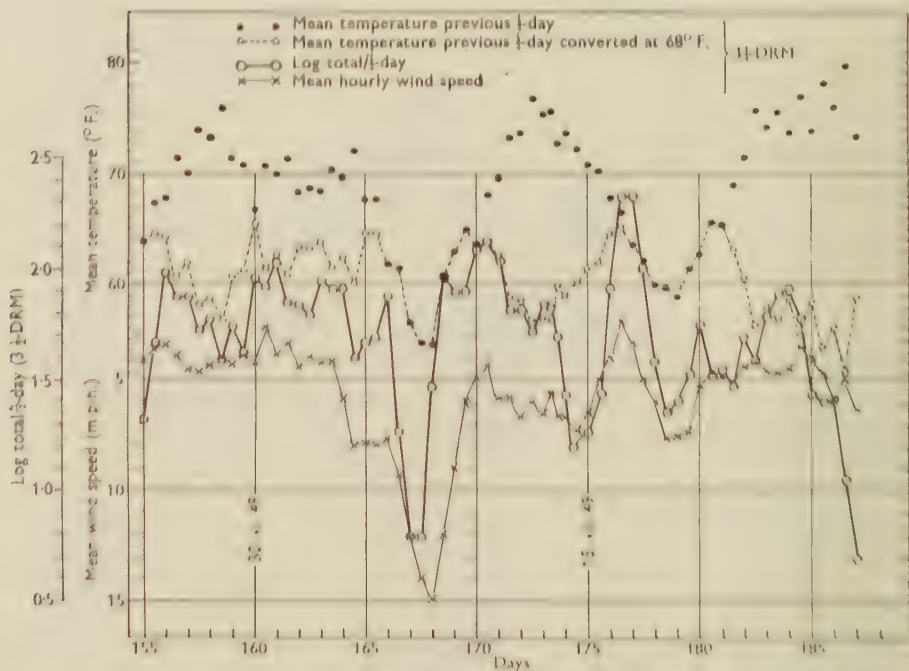


Fig. 6. Trap B. The relation between the 3 half-day running means of log total $\frac{1}{2}$ -day, mean wind-speed, mean temperature of previous $\frac{1}{2}$ -day and mean temperature converted at an arbitrary optimum of 68 $^{\circ}$ F.

Statistical analysis of field data alone, however, without controlled experiments, cannot show if the optimum is spurious or peculiar to the present data or if it is of biological significance; for even if it occurs generally it may be masked in nature.

Similarly with the effect of temperatures of the same and of the previous half-day. Each is statistically significant when considered without the other, but neither are significant if included together in a multiple regression (Table 7).

TABLE 7. *Multiple regressions of log total/½-D on temperature and wind-speed: trap B, 1949*

b =partial regression coefficient for log total/½-D on x_{1-4} and z .

x_1 =mean temperature, ° F. for same ½-D, $x_2=(x_1)^2$.

x_3 =mean temperature, ° F. for previous ½-D, $x_4=(x_3)^2$.

z =mean wind-speed, m.p.h. n =number of observations.

ss =sum of squares for regressions as % total sum of squares.

	b	S.E.	$P=0.05$	ss	n
Primary data					
x_1	0.3937	0.1410	Sig.		
x_2	-0.0028	0.0010	Sig.	22%	68
z	-0.0158	0.0222	Not sig.		
3 ½-DRM					
x_1	0.3159	0.1390	Sig.		
x_2	-0.0024	0.0010	Sig.	31%	66
z	-0.0552	0.0191	Sig.		
x_3	0.0365	0.0112	Sig.	25%	66
z	-0.0319	0.0152	Sig.		
(x_2 converted at 66° F.)					
x_2	0.0443	0.0131	Sig.		
z	-0.0138	0.0552	Not sig.	26%	66
(x_3 converted at 68° F.)					
x_1	0.2600	0.1406	Not sig.		
x_2	-0.0018	0.0010	Not sig.		
x_3	0.2066	0.1632	Not sig.	40%	66
x_4	-0.0017	0.0012	Not sig.		
z	-0.0496	0.0223	Sig.		

and although the significance tests show the effects of each temperature to be due to more than chance, they give no indication of their biological reality, for because the temperatures are correlated with each other, both will show correlations with catch even if only one is operative.

The regressions of temperature and wind-speed on numbers flying

A multiple regression was calculated for the primary data of log total/½-D on a quadratic function of temperature, and on a linear function of wind-speed both for the same half-day. The partial regression coefficient for temperature is statistically significant, that for wind-speed is not (Table 7). Thus apparently it is low temperature rather than high wind (with which it is associated, Fig. 7), which lowers the catch. However, only 22% of the variation in log total/½-D (as expressed by

sums of squares) is accounted for by the combined regressions for current temperature and wind-speed changes.

A similar multiple regression, but with running means ($3\frac{1}{2}$ -DRM) shows significant partial regressions with both wind-speed and temperature (Table 7). Besides being significant, the regression coefficient for wind-speed is much larger than with the primary data; i.e. not only is the relationship less likely to be due to chance, but for the same change in wind-speed the amount of change of log catch is greater with running means than with the primary data. However, only 33 % of sums of squares is accounted for by the regressions.

If with running means, the temperature of the previous half-day is included in a 5-fold regression (Table 7), 40 % of the variation in log total/ $\frac{1}{2}$ -D is accounted for, although of all the factors only wind-speed is statistically significant. But although the individual regressions for temperature are not significant there is little doubt that they are significant collectively.

CHANGES IN POPULATION AND BEHAVIOUR AND STATISTICAL METHODS USED TO DISTINGUISH THEM

The question arises, which of the changes in numbers of aphids flying are due to population fluctuations and which to variations of flight behaviour? Williams (1940) writes of insects in general: 'The activity varies rapidly from hour to hour and from night to night and is determined largely by the weather conditions of the moment. . . . The population changes happen more slowly. . . . Thus if one considers the difference in catch between two successive nights, the effect will be largely due to activity; whilst the differences in catch between two similar nights in different months will be largely due to population'.

For a statistical analysis on these lines it is usual to express activity, i.e. behaviour changes, either by the difference between the successive log catches (equivalent to the ratios of actual catches) or by differences of each log catch from the running means; the running mean of log catch itself is considered to reflect population change (Williams, 1940). Such an analysis was attempted with the *A. fabae* data. It has been shown, however (pp. 531-2 above), that differences of successive log hourly catches of *A. fabae* are not related to wind-speed changes as would be expected if they reflected changes in flight behaviour. Deviations from running means also are not significantly related to wind-speed. Moreover, if the running means reflected mainly population change one would not expect them to be related to wind-speed, whereas, in fact, they are (Table 7). On the other hand, since the running means themselves are of aphids actually flying it cannot be expected that these changes would be divorced from behaviour effects. It seemed therefore that the method was based on false assumptions; and arithmetical models, in which changes were arranged to represent population and behaviour fluctuations, showed that both the differences in successive log catches and deviations from a running mean will reflect *either* population *or* behaviour changes according to which

makes the greater contribution to changes in the catch. It is similar with the running mean itself. Thus there must be a considerable advance in knowledge of the relative rates of population and behaviour change before they can be further analysed with these methods. With aphids, at any rate, it is at present impracticable to assign in this way so much change to population, so much to behaviour factors. Changes in the percentage flying cannot be calculated merely from changes in aerial numbers, for the population on the crop capable of flight must also be known: and because aphids leave the locality, population itself is affected by activity.

METEOROLOGICAL FACTORS OTHER THAN TEMPERATURE AND WIND-SPEED

Rainfall. Scarcely any rain fell during the whole trapping period. On no occasion was the effect of rain on catch obvious and the data are too few for statistical analysis.

Hours of sunshine. No correlation between hours of sunshine and $\frac{1}{2}$ -day catches could be detected with trap B.

Wind direction. Although the main sources of *A. fabae* close to trap B were to the north and south-west no correlation of numbers caught with wind direction was apparent.

Atmospheric humidity. The humidity as measured in this work only indicated the general dampness or dryness; but this is what previous workers have considered and for which effects have been claimed (Maldwyn Davies, 1935; Thomas & Vevai, 1940; Broadbent, Chaudhuri & Kapica, 1950). Maldwyn Davies (1935, 1936) thought that low relative humidity encouraged aphid flight; Broadbent (1947) records that high humidities do not prevent take-off and indicated (1949) that it is *changes* in humidity which affect flight activity. It would, however, be surprising to find it associated with hour-to-hour density changes even if it affected take-off strongly, for reasons similar to those mentioned on pp. 531-2 with reference to wind-speed.

Nevertheless the following relations have been considered:

Differences of log catch from hour to hour with

(1) Mean hourly saturation deficit (trap A).

(2) Hour-to-hour *differences* of mean hourly % R.H. (trap B).

Log total no./ $\frac{1}{2}$ -day (trap B) with

(3) Mean saturation deficit/ $\frac{1}{2}$ -day.

(4) Mean % R.H./ $\frac{1}{2}$ -day.

3 $\frac{1}{2}$ -DRM of log total no./ $\frac{1}{2}$ -day (trap B) with

(5) 3 $\frac{1}{2}$ -DRM of saturation deficit.

In none of these were significant correlations observed, although in (1) and (2) the tendencies were towards negative relations.

Partial correlations of density change with humidity in association with temperature and wind-speed and their changes have not been made; nor has change of humidity from one ' $\frac{1}{2}$ -day' to another been tested, since the effects of humidity

change appear to be rapid (Broadbent, 1949) and likely to take effect within rather than between ' $\frac{1}{2}$ -day' periods.

The fact that the highest catches were made at *high* humidities (Fig. 7) does not indicate its importance, since the occasions were also fairly calm. It is also very difficult, in the field, to dissociate the effects of humidity and temperature since the two factors tend to vary reciprocally. Thus the low activity attributed by Muller & Unger (1951) to high humidity could equally well have been due to the associated low temperature.

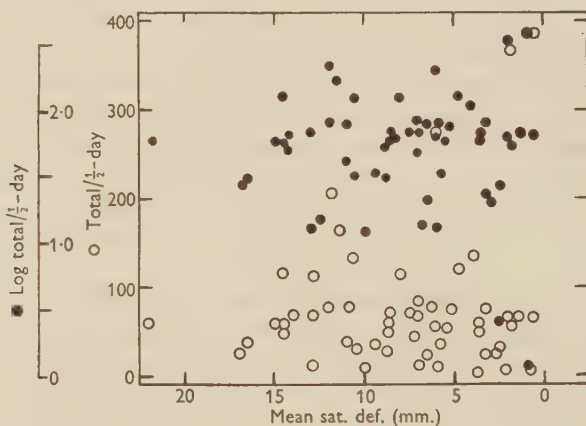


Fig. 7. Trap B. Total/ $\frac{1}{2}$ -day and log total/ $\frac{1}{2}$ -day in relation to mean saturation deficiency.

THE COMPARISON OF DATA FROM TRAPS A AND B

Data from trap A failed to show significant relationships of catch and weather due to masking effects of large population changes. The only major departure from an otherwise fairly smooth curve which could be associated with weather was on 7 July (day 167), when, as in trap B, numbers dropped almost to zero after extremely high values on the preceding day. It is unlikely that wind alone was responsible for this, for a much greater catch of 120/hour was made in trap A at the same wind-speed, 3.5 m.p.h., on day 161 even before the population was at its maximum. Moreover, there was a recovery in trap B to a high catch on days 168 and 169 in spite of high winds (Table 6). Temperatures, however, were very low at trap A on day 167 and little above the flight threshold (Table 5), which may be regarded as lying between 7–8° C. (44.6–46.4° F.), ambient temperature, the lowest at which flight began on any morning. On the other hand, higher hourly catches than on day 167 were obtained on days 168 and 178 at the same hourly temperatures. It is possible therefore that the drop on day 167 was due not only to the temperatures of the occasions but to the gradual lowering of temperature in the preceding three days. A 'piling up' of later instars or alates over this period could have produced high densities subsequently even if the proportion flying was not maximal.

TOTAL NUMBERS FLYING AT DIFFERENT WIND-SPEEDS

If aphids take-off in lulls within half-day periods then a false relation may be given by correlating numbers flying with mean wind-speed for half-day periods. Hourly values have therefore been examined in order to minimize the effects of lulls, and since trap B was 50-60 ft. from the crop it is unlikely that aphids were trapped only after immediately taking off in lulls.

TABLE 8. *Hourly catches in trap B grouped under mean hourly wind-speeds*

Mean wind-speed (m.p.h.)	No. of hours with catch	Total* catch	Max. hourly* catch
0-	13	109.0	23.0
1-	20	311.0	89.9
2-	54	659.0	72.4
3-	53	588.9	93.8
4-	59	494.9	73.1
5-	69	658.6	65.5
6-	49	530.8	47.7
7-	42	481.3	76.7
8-	36	216.4	20.0
9-	26	212.4	31.6
10-	14	62.6	15.3
11-	7	53.0	18.5
12-	6	59.8	19.5
13-	6	97.6	34.5
14-	2	39.3	22.4
15-	1	3.3	—
16-	2	9.0	—
17-	—	—	—
18-	2	16.8	14.4
19-	2	43.3	26.0

* Corrected for wind-speed (Table 1).

All hourly catches at trap B, which was the most exposed trap, were therefore grouped under their corresponding mean hourly wind-speeds: zero catches in the daytime were negligible and were omitted. Table 8 shows that for the summer season of continuous (not intermittent) trapping, 77 % of all the *A. fabae* trapped were caught when the mean hourly wind-speed was over 3 m.p.h., the speed beyond which there is little or no voluntary control over flight direction; and 53 % of the total were caught above even 5 m.p.h.

The trap measures density/hour; assuming that the mean hourly wind-speed represents conditions when aphids were actively in flight, the number delivered into a constant vertical area would be much greater than 77 or 53 %, for each hourly density must be multiplied by the appropriate wind-speed. The numbers delivered by winds above 3 m.p.h. would then be 93 %, and above 5 m.p.h., 78 % of the total.

DENSITY CHANGE IN RELATION TO MOULTING RATES, THE PERIOD BETWEEN MOULTING AND THE FIRST FLIGHTS, AND A POSSIBLE EXPLANATION OF THE DAILY DOUBLE PEAK

The results of a limited amount of work suggest that the explanation of the double peak of density within the day may be found in the relation of moulting to flying times and in the circumstances which allow newly moulted alates to accumulate before take-off; this depends on maturation, not only on flight conditions.

TABLE 9. *Numbers moulting into alatae at different times of day*

	Time	No. moulted on three plants	Temp. (° F.)		Time	No. moulted on three plants (new plants)	Temp. (° F.)
17 Aug.	Overnight	0	—	18 Aug.	Overnight	8	55 (min.)
	06.20	25	61		05.55	16	59
	07.30	47	72		06.40	59	65
	09.20	39	81		07.40-09.20	26	66
	10.20	21	80		09.20	12	67
	11.20	14	83		10.15	14	68
	12.20	18	82		11.15	16	68
	13.20	23	84		12.15	11	68
	14.20	22	83		13.15	8	67
	15.20	16	84		14.15	12	68
	16.20	20	83		15.15	7	69
	17.20	19	80		17.15	17	71
	18.20-20.40	41	68				
	20.40	7	61				

Of the winged nymphs observed to moult towards the evening, few have flown before the next morning; their accumulation overnight could provide insects for the first density peak in the morning, whose subsequent decline could be due to depletion of alatae in a condition to fly. Also while moulting may cease during the low night temperatures there may be a consistent peak of *numbers* moulting (if not of proportion moulting) in the early morning in spite of a relatively low temperature (Table 9). This may explain the 'optimum' at 68° F. Such alates of the same age have been observed to fly at about the same time some hours later and could constitute the second density peak.

This hypothesis, however, requires a good deal of further study.

Times between moulting and flying

The time between moulting and the first flight has been observed to vary from about 2 hr. to at least 30 hr.

Seven aphids newly moulted into alates in the morning were marked with a minute spot of red water-colour paint on the thorax and kept under almost continuous observation. In spite of vigilance the actual act of flight was not observed with any of the marked insects, but that they flew is certain, since they could not have walked far without being detected.

The marked aphids all departed within a fairly short space of time ($26\frac{1}{2}$ – $29\frac{1}{4}$ hr.

after moulting) which would indicate a peak of aerial density. Temperatures over the whole period were fairly low ($61-71.5^{\circ}$ F. during the first day, 48° F. night minimum) and no doubt accounted for the long period between moulting and flying, and a suction trap a few feet away showed very low densities over this period in spite of a fairly high alate population on the crop.

During the period between moulting and flying the alates usually spend a considerable time with the proboscis inserted, presumably feeding.

CONCLUSIONS

The regressions of current wind-speed and temperature account for only a small proportion of the change in catch; and although it is known that wind-speed exerts a strong influence on the take-off behaviour of individual aphids it cannot yet be claimed with certainty that it is even a major factor influencing numbers in the air. For whether wind-speed appears to be of significance in the collective picture (as distinct from its effect on individuals) depends on how the data are treated. With the primary data wind-speed is neither significant nor with a strong effect, but the reverse is true with running means and no explanation can be given for this at present.

Apart from this, the true effects of wind on behaviour in the field are not likely to be simple, for during the day the effects of wind and temperature are opposed. As temperature rises in the morning and encourages flight, so wind-speed also rises and tends to discourage it; the reverse occurs in the evening. Yet from one day to another the effects of wind and temperature enhance each other for cold days are usually the windy ones (cf. within and between days, Fig. 1).

However, whether it is wind or temperature or both which are operative, their combined effects are shown by the regression equations. With the $3\frac{1}{2}$ -DRM's of $\log \text{total}/\frac{1}{2}\text{-D}$ and wind-speed, the partial regression coefficients are -0.0552 and -0.0496 respectively. That is, with the first coefficient, 1 m.p.h. rise in the running mean of wind-speed is associated with an average drop of 0.0552 in the running mean of $\log \text{total}/\frac{1}{2}\text{-D}$; and for a fall of 1 m.p.h. there is a rise of 0.0552 . Thus with running means, for a rise of 5 m.p.h. in the wind-speed the *actual* (not *log*) $\text{total}/\frac{1}{2}\text{-D}$ drops by a geometric average of 1.88 times—i.e. $\text{antilog}(5 \times 0.0552)$. For the coefficient -0.0496 the equivalent figure is 1.77. In view of this, to state that only a small proportion of the total variation in numbers flying is accounted for by wind and temperature changes may seem a contradiction requiring explanation.

The 1.88-fold increase in catch for a drop of 5 m.p.h. in wind is an average (geometric) trend represented by the best-fitting straight line drawn through the scattered points. But observations show so many great departures from this average that any one change in wind-speed is unlikely to be associated with an average effect on the catch; indeed, a drop in wind may even be accompanied by a fall in catch and vice versa. For example, 50 % take-off from 300 aphids on one

day produces a larger flight than 100 % take-off from 100 aphids on the following day. Thus of the total amount of change shown by all the data, only 22-33 % is associated with the combined changes in both current wind-speed and temperature, as shown by the general slope of the data (the regression line).

With the primary data, the formula for the average effects of current temperature and wind-speed (Table 7) is

$$y = -12.1313 + 0.3937x - 0.0028x^2 - 0.0158z,$$

for the running mean

$$y = -8.3608 + 0.3159x - 0.0024x^2 - 0.0552z,$$

where y is expected $\log \text{total}/\frac{1}{2}\text{-D}$, x is temperature ($^{\circ}\text{F.}$) and z is wind-speed (m.p.h.). From y the actual expected geometric mean catch can be obtained by antilogs.

The reason why so much variation remains unaccounted for may be due to other natural factors besides wind-speed and temperature, or to errors of measurement, sampling errors and to the assumption of linearity for curvilinear relations although this was not evident with logarithms. Humidity, sunshine, rainfall and wind direction appear to have no measurable effects with the present data (p. 541). But population changes are of major importance in causing rapid changes in aerial numbers and probably outweigh most of these other factors.

If alatae accumulate during a windy period and then fly off in great numbers when the wind drops it may be thought that numbers flying depend on wind-speed. But this is to confuse the behaviour and population factors. The number of alatae which fly is due to a population change; whether they accumulate or not may make no difference to the percentage which take-off due to wind-speed. Thus the accumulation is also a population change, and the wind merely determines when they will fly, rather than the ultimate numbers which do so. Thus wind-speed may affect the occurrence of high concentrations but not necessarily the amount of migration over a considerable time.

But in any case, high winds do not always inhibit flight. On day 168 (Fig. 6, Table 6) numbers increased while the wind was at its maximum, and this suggests that an accumulation of aphids in a limited area of leaf or stem may itself be a factor encouraging movement and flight even in spite of adverse weather conditions.

Thus the decline in aerial densities by lowered take-off rates and depletion and their increase by high take-off rates following accumulation has been represented by a single regression graph which can be read in either direction. But it would be a mistake to interpret this as the positive and negative reaction of a single simple process. The regression does not show a relation between a series of independent, isolated events, each of which can be repeated on another occasion. Though not shown by the regression graph, the points are in fact interdependent, for each has followed from another in a time sequence in a manner peculiar to the present data.

A drop in density due to low temperature is not, as far as one knows, because the process of density rise has been reversed and insects fall back on to the crop, but presumably because the numbers of take-offs diminish. Moreover, a density, being a resultant of take-off rate from the crop and departure rate from the local air, is determined by at least these two actions, both of which could vary independently with temperature. On another occasion or in another place the regressions may be quite different—as at trap A where they are too small to be significant.

It is a pleasure to thank Mr J. Dunwoody for help and advice in statistical matters, Mr L. R. Taylor for help in the field and elsewhere and the staff of the Physics Department for meteorological records.

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THE PENETRATION OF THE INSECT CUTICLE BY DDT AND RELATED COMPOUNDS

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(With 2 Text-figures)

The penetration of *pp'*-DDT and some related compounds into grain weevils has been studied by a micro-analytical technique.

Grain weevils were exposed to filter papers, impregnated with the chemical under test, for periods varying from a few hours to several days. The chemical removed by washing the insects with cold methanol, which was not expected to dissolve anything which had penetrated below the wax layer of the epicuticle, was described as being 'outside' the insect; that which remained was extracted with ether after grinding the insects with anhydrous sodium sulphate and recorded as being 'inside' the insect. By plotting the amounts 'inside' and 'outside' after varying times of exposure a picture of the rate of penetration of these compounds was obtained.

The results are discussed in relation to similar curves published earlier for isomers of benzene hexachloride. The major difference revealed is the very slow penetration of *pp'*-DDT compared with γ -BHC. This is ascribed to the vapour effect of γ -BHC which has a vapour pressure much greater than that of *pp'*-DDT.

INTRODUCTION

In a recent paper from this laboratory (Armstrong, Bradbury & Standen, 1951) the penetration of the cuticle of the grain weevil (*Calandra granaria*) by isomers of benzene hexachloride was described. There it was shown that grain weevils exposed to deposits of benzene hexachloride on filter papers take up insecticide through the waxy covering of the epicuticle, which first becomes saturated with the chemical, and that penetration into the inner tissues follows at a slower rate which varies with the structure of the isomer concerned, being most rapid for γ -benzene hexachloride.

The work we now describe with *pp'*-DDT was undertaken partly to gain information on the mode of action of this insecticide and partly to throw more light on the meaning of the penetration curves already established for the isomers of BHC. Curves for the active insecticide *pp'*-DDT were compared with those for the inactive or only slightly active related compounds *op'*-DDT, *pp'*-DDE and *op'*-DDE.

METHOD

The experimental method used was very similar to that described in the earlier paper (Armstrong *et al.* 1951). *C. granaria* were used as test insects and these were kept in contact with filter-paper impregnated with the chemical at approximately $110\mu\text{g./cm.}^2$ for varying periods of time. (The dosage is ten times that used for

BHC isomers; it was increased to keep in line with practical application rates for *pp'*-DDT.) The chemical picked up was estimated in two fractions one, 'outside', obtained by methanol washing the whole insects and the second, 'inside', by crushing the insects in a mortar with anhydrous sodium sulphate and extracting with cold ether or carbon tetrachloride. This is essentially the method employed by Sternburg, Kearns & Bruce (1950) in their study of the absorption of DDT by houseflies. For BHC we used nitric acid to set free 'inside' chemical but this was not possible with DDT as it reacts with this reagent.

The analytical method was the colorimetric one described by Schechter, Soloway, Hayes & Haller (1945) and is based on nitration followed by treatment with alkali. All four compounds give colours by this reaction and by slight modification of the conditions for *pp'*-DDT quantitative methods for *op'*-DDT, *pp'*-DDE and *op'*-DDE were obtained.

EXPERIMENTAL PROCEDURE

Preparation of compounds for test

The compounds chosen for the experiments were 1:1-di-(*p*-chlorophenyl)-2:2:2-trichloroethane (*pp'*-DDT), 1-(*p*-chlorophenyl)-1-(*o*-chlorophenyl)-2:2:2-trichloroethane (*op'*-DDT), 1:1-di-(*p*-chlorophenyl)-2:2-dichloroethylene (*pp'*-DDE) and 1-(*p*-chlorophenyl)-1-(*o*-chlorophenyl)-2:2-dichloroethylene (*op'*-DDE).

Pure samples of *pp'*-DDT and *op'*-DDT were isolated from commercial DDT by the method described by Haller and others (1945). The corresponding ethylenic compounds (*pp'*-DDE and *op'*-DDE) were prepared from the DDT compounds by treatment with alcoholic potash. The identity of the four samples was checked by melting-point determinations.

Exposure of the insects to the chemicals

15 cm. diameter no. 1 Whatman filter-paper was impregnated with 2 ml. of 1% acetone solution of the chemical, evenly distributed over the surface from a pipette, and the solvent allowed to evaporate. The treated paper was placed in the bottom of a Petri dish, 2½ g. of mature grain weevils placed on top of the paper and the whole covered with the upper portion of the Petri dish. The insects were allowed to remain in contact with the paper for varying periods of time in a constant temperature and humidity room (temperature range 74–80° F., percentage relative humidity 60–72) before their removal for analysis.

Removal of chemicals from the insects for analysis

Insects which had been exposed to the chemical in the way described above were removed from the filter-paper, separated from the frass, and placed in a separating funnel over a pad of glass wool. In this vessel they were quickly rinsed with three 10 ml. quantities of methanol, the washings being collected together, evaporated to small bulk on the water-bath and finally air-blown to dryness in a stream of air

which had been dried by passing through a tower containing pellets of potash. The chemical isolated as a result of these washings was determined and recorded as being 'outside' the insect cuticle.

The washed insects were ground with anhydrous sodium sulphate in a pestle and mortar until they were finely subdivided and then extracted four times with cold ether or carbon tetrachloride. The extracts were filtered through a Whatman no. 41 paper, collected together, and the solvent removed by evaporation and air blowing as described above. The chemical isolated by this treatment is described as being 'inside' the insect cuticle.

Before attempting to determine the amount of chemical isolated from 'inside' the insect it was necessary to remove the fat extracted by the ether. The method first attempted was that described by Schechter, Pogorelskin & Haller (1947) in which a carbon tetrachloride solution of the chemical and fat is given a prolonged treatment with fuming sulphuric acid in a separating funnel. It was found subsequently that this unpleasant and time-consuming operation could be successfully avoided by modification of the method used by Davidow (1950) for the separation of DDT from fat in the analysis of DDT in milk samples. The details of this modified process are as follows.

5 g. of a high grade kieselguhr (Celite Filtercel and Kensil P 11 were used in our experiments) was mixed intimately in a mortar with 3 ml. of a mixture of equal parts of 60% oleum and sulphuric acid to give a damp powder; sufficient carbon tetrachloride to form a stiff slurry was then incorporated in the mixture. 10 ml. of carbon tetrachloride was introduced into a column 2 cm. diameter and 16 cm. long, closed at the lower end with a sintered glass plug, and sufficient of the kieselguhr slurry added to give a column height of about 5 cm. The column was then gently packed with a glass tamping rod to give a uniform appearance and texture.

The mixed chemical and fat was dissolved in 10 ml. of carbon tetrachloride and the solution introduced into the top of the column; its downward passage was assisted by very slight suction if the rate of flow was appreciably less than 120 drops per minute. The column was washed through with two further 10 ml. quantities of carbon tetrachloride and finally with 15 ml. of the same solvent added from a pipette so as to wash the walls of the column. (It is important to add the carbon tetrachloride gently without disturbing the surface of the kieselguhr and thus cracking the column.) The eluate was freed from solvent by evaporation on the water-bath, the last few ml. being removed by blowing with a stream of dry air at laboratory temperature.

Analytical method

(1) *pp'*-DDT

The method used was the colorimetric method which depends on nitration followed by the development of a colour by treatment with caustic alkali (Schechter *et al.* 1945).

To the dry extract (freed from fat as described above in the case of the 'inside'

determinations) was added 5 ml. of a 0.2% carbon tetrachloride solution of oleic acid and the solvent removed. 5 ml. of nitrating mixture (1 vol. H_2SO_4 + 1 vol. fuming HNO_3) was then added and nitration continued under reflux on a water-bath for 60–90 min. After removal from the water-bath and cooling 20 ml. of water was added down the condenser and the solution boiled to remove nitrous fumes. The flask was again cooled and a further 20 ml. of water added. The product was extracted three times with a mixture of one part of diethyl ether and four parts of petroleum ether (60–80° C.) using 15, 10 and 5 ml. quantities. The combined extract was washed with 2% aqueous sodium hydroxide and again with saturated sodium chloride solution (3 ml.). The washed extract was heated on a water-bath until completely free of solvent and water. After cooling, 5 ml. of benzene was added followed by 10 ml. of 5% alcoholic potassium hydroxide to develop the blue colour. A 'blank' was also prepared by mixing the same volumes of benzene and alcoholic potassium hydroxide. After allowing to stand for 10 min. the two solutions were filtered through no. 41 Whatman papers into 1 cm. quartz cells and the colours compared in a Hilger Spekker photometer using the Spectrum Yellow filter no. 606.

The amount of DDT corresponding to the Spekker reading was read off from a calibration curve. The calibration curve was prepared by working through the whole process with insects—the known amounts of DDT being added to the insects in the separating funnel prior to washing for the 'outside' determinations and to the ground insect-sodium sulphate mixture for the 'inside' determinations. The points obtained for both 'outside' and 'inside' determinations all lay on the same straight line, giving one calibration curve for the two treatments.

(2) *op'*-DDT

To determine *op'*-DDT the procedure was the same as for *pp'*-DDT up to the stage where the solution is set aside to develop the colour. It was found that the red-blue colour produced by *op'*-DDT reached a maximum in 2 min. and then faded rapidly. More consistent Spekker readings could be obtained by allowing the solutions to stand for 19 hr. by which time a deep yellow colour had been produced, which could be measured in the Spekker using a Spectrum Violet no. 601 filter.

Calibration was done as described for *pp'*-DDT and again both 'outside' and 'inside' determination of known quantities gave points on one line.

(3) *pp'*-DDE

The determination of *pp'*-DDE followed closely the method for *pp'*-DDT but it was found necessary to include an extra 15 ml. carbon tetrachloride wash to complete the removal of this compound from the kieselguhr-sulphuric acid column. The red colour produced by the alcoholic potash reached a maximum after 65 min. and was then measured using a Spectrum Yellow-Green no. 605 filter. Calibration points for both 'inside' and 'outside' determinations again lay on one straight line.

(4) *op'*-DDE

The method was as described for *pp'*-DDE. The red-blue colour resulting from treatment with potash reached a maximum in 17 min. and was measured using the Spectrum Blue-Green no. 603 filter. Calibration points for 'outside' and 'inside' lay on the same straight line.

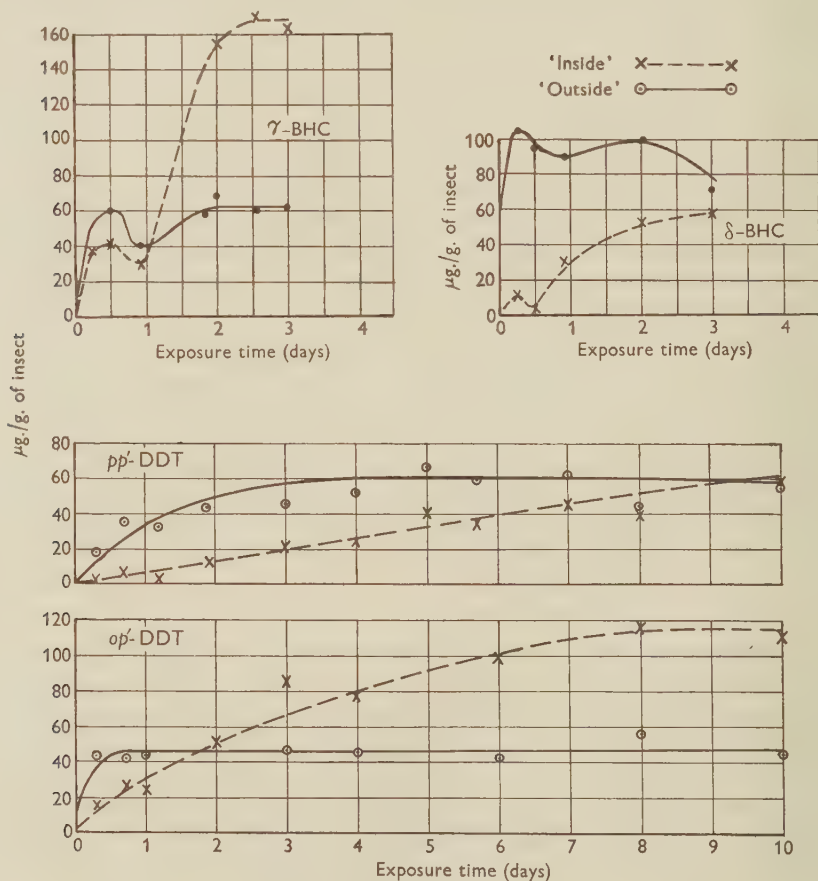


Fig. 1. Penetration of DDT and BHC into grain weevils.

RESULTS

The results are expressed graphically in Figs. 1 and 2, where the amounts of chemical found 'inside' and 'outside' have been plotted in terms of $\mu\text{g./g.}$ of insect against time of exposure to treated paper. For each of the four chemicals the best 'inside' curve is shown as a dotted line and the best 'outside' curve as a full line. Each point is the mean of at least two determinations.

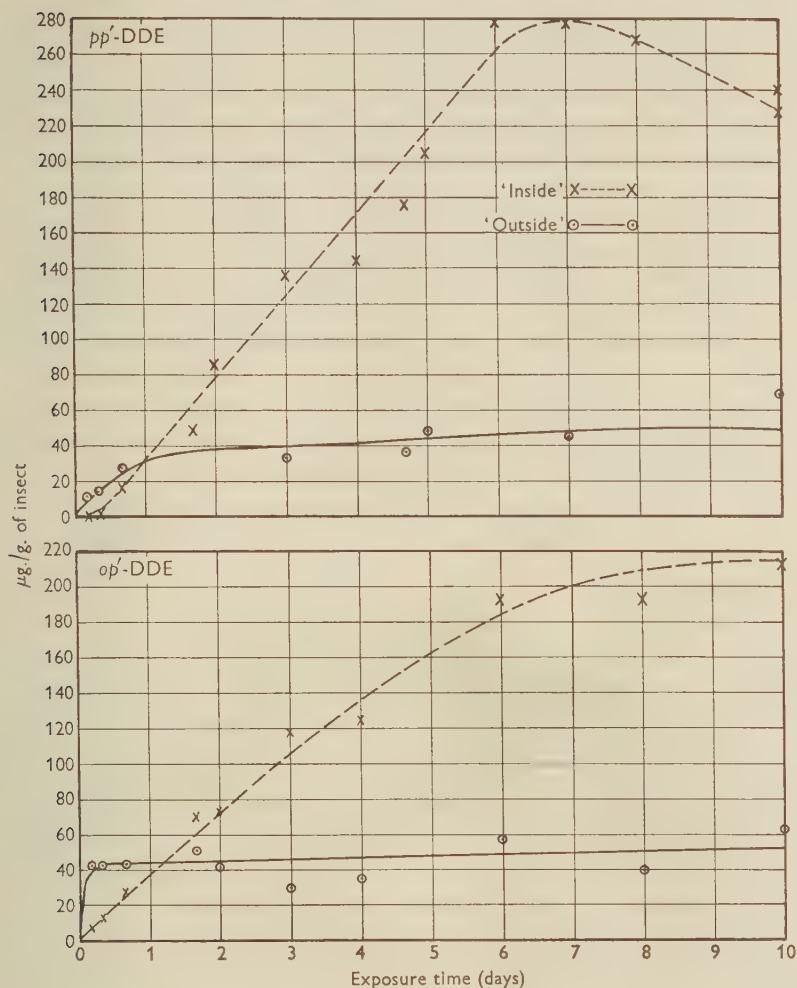


Fig. 2. Penetration of DDE into grain weevils.

DISCUSSION

It is instructive to consider the DDT curves in connexion with those previously published (Armstrong *et al.* 1951) for the BHC isomers. To facilitate the comparison the penetration curves for γ - and for δ -BHC have been reproduced on the same scale in Fig. 1.

It is evident that the DDT family of curves presents a very different picture from that of the BHC isomers.

The most striking difference is the much slower succession of events in the *pp'*-DDT curve compared with γ -BHC; thus for *pp'*-DDT the 'outside' curve reaches a maximum of approximately 60 $\mu\text{g./g.}$ in 3-4 days whereas γ -BHC reaches the same maximum in 12 hr. 'Inside', also, the value of 40 $\mu\text{g./g.}$ is reached in 12 hr. by γ -BHC whereas *pp'*-DDT requires 6 days to reach this figure.

On the other hand the compounds structurally related to *pp'*-DDT reach equilibrium 'outside' values fairly rapidly (less than 1 day) and the amount 'inside' rises steeply, especially for the two unsaturated compounds; although even these do not reach high values in so short a time as γ -BHC.

Thus the picture for the BHC isomers, where the insecticidally active member is the only one to show rapid penetration into the insect inner tissues, is reversed in the DDT series where the active compound shows least rapid penetration. The conclusion must be drawn that although structure profoundly affects rate of penetration of contact insecticides lack of ability to penetrate rapidly does not preclude insecticidal activity. The rate of penetration must of course depend both on the insect studied and the conditions of the exposure. Barlow & Hadaway (1952)

TABLE 1. *Solubilities in paraffin and maximum values of 'outside' curves*

	<i>pp'</i> -DDT	<i>op'</i> -DDT	<i>pp'</i> -DDE	<i>op'</i> -DDE	γ -BHC	δ -BHC
Solubility at 24° C. in (light spray oil) g./100 g. solvent	4.0	17	9.3	8.6	5.0	12.7
Maximum value of 'out- side' curve $\mu\text{g./g.}$ insect	60	45	60	50	61	102

have recently published work on the pick-up of DDT by mosquitoes from fine particle size deposits on various porous surfaces. Although these authors did not determine the 'inside' DDT directly, it appears from the figures that under their conditions *pp'*-DDT penetrates the insect cuticle very much more rapidly than in our experiments with grain weevils. The failure of DDT to penetrate grain weevils rapidly is reflected in the slowness of its action on these insects; in our experiments for instance γ -BHC at 11 $\mu\text{g./cm.}^2$ on the filter paper gives 100% knock-down in a few hours whereas DDT at 110 $\mu\text{g./cm.}^2$ requires 3-4 days.

No relationship could be found between the maximum values of the 'outside' curves and the solubilities of the DDT family of compounds in paraffin recorded in Table 1.

For the BHC isomers there seemed to be a parallel between the solubility in light spray oil and the maximum 'outside' value and this led to the hypothesis that the first step in penetration was saturation of the outer wax layer of epicuticle by the chemical. If the relative solubility of the compounds in paraffin is regarded as an indication of their relative solubilities in the wax of the epicuticle it is clear that only *pp'*-DDT can approach saturation, and the related compounds must fail to reach saturation in the wax layer of the epicuticle.

If the wax layer of the epicuticle is a reservoir which is drained by movement towards inner tissues at the same time as it is replenished by further supplies from

the substrate or surrounding vapour, it is evident that the level of concentration of chemical in the wax layer will only approach saturation if penetration is slow compared with pick-up. The level of the 'outside' curve may therefore represent a steady state determined by the relative rate of movement of chemical into and out of the epicuticle wax layer.

It is quite likely that the more rapid initial stages in the penetration of γ -BHC are related to its relatively high vapour pressure. γ -BHC has a vapour pressure much greater than that of pp' -DDT (Shepard (1951) quotes 9.4×10^{-6} mm. Hg for γ -BHC and 1.5×10^{-7} mm. Hg for pp' -DDT at 20° C.) and its solution over the whole insect epicuticle by absorption from the vapour phase will be greatly facilitated compared with pp' -DDT, which may have to rely to a much greater extent on direct contact between solid deposit and insect tarsi.

TABLE 2. Vapour effect of insecticides

	Amount determined (μ g./g.)			
	pp' -DDT		γ -BHC	
	'Outside'	'Inside'	'Outside'	'Inside'
Insects in contact with treated surface (average values from the curves of Fig. 1)	27	4	38	42
Insects separated from treated surface by clean filter-paper (average of two experiments)	4	1	55	59

The importance of this difference was brought out when direct contact with the deposit was prevented by placing the insects on an untreated filter-paper over the treated one. Results for pp' -DDT and γ -BHC are given in Table 2.

It is clear that γ -BHC is not picked up to any less extent when direct contact with solid deposit is prevented whereas pp' -DDT, although it appears to have some vapour effect, is picked up only in very small amount.

Important differences in the behaviour of flies on γ -BHC- and DDT-treated paper surfaces have been observed by Baranyovits (1951) who has also stressed the importance of the vapour effect of γ -BHC (1951, private communication).

The work of Hadaway & Barlow (1951) has also shown a difference between the behaviour of BHC and DDT deposits which may be traced to vapour effect. These authors showed that DDT and BHC water dispersions sprayed on to mud surfaces differed greatly in persistence, the BHC remaining active under these conditions much longer than DDT. The fine particles of BHC appear to be carried into the capillaries of the mud surface, where they constitute a reservoir of insecticidal potency because the vapour pressure of BHC is sufficiently high for it to act at a distance, whereas pp' -DDT so carried from the surface would lose its insecticidal action.

The rapid penetration of the unsaturated compounds pp' -DDE and op' -DDE cannot be explained. It was thought that the unsaturated nature of these compounds

might give them some surface activity making them penetrate by a surface diffusion mechanism such as that envisaged by Hurst (1943) but when mixed solutions of grain weevil wax, isolated as described in our earlier paper (Armstrong *et al.* 1951), and *pp'*-DDE were spread on water the film pressure/area curve determined by the surface film balance (Harkins & Anderson, 1937) was that of the original wax. The unsaturated compounds were not surface active and did not appear to interact with the insect wax film.

The authors are grateful to their colleagues at Hawthorndale Laboratories, and particularly to Dr F. Baranyovits, for valuable criticism and suggestions, and to Dr V. B. Wigglesworth and Dr J. W. L. Beament, who provided valuable ideas in a preliminary discussion.

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THE USE OF TETRA- AND PENTA-CHLORONITRO-BENZENES IN THE CONTROL OF *BOTRYTIS* DISEASE AND *RHIZOCTONIA* ATTACK OF LETTUCE

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(With 1 Text-figure)

Botrytis disease in the 'spring' lettuce crop was controlled by six talc dusts containing 5, 10 or 20 % of tetra- or penta-chloronitrobenzene. All the dusts increased equally the final stand in the frames, but the number of seedlings with stem lesions decreased with increasing concentrations of both compounds. At similar concentrations the tetra- was more effective than the penta-compound. The percentage survival in the field was increased by the dust treatments. The 20 % dust of the penta- was only as effective as the 5 % dust of the tetra-compound.

The dusts affected the leaves, stunted the seedlings and delayed hearting. At similar concentrations the tetra- was more harmful than the penta-compound. The 5 % dust of the tetra- was less damaging than the 20 % dust of the penta-compound.

The spread of *Rhizoctonia solani* was reduced by incorporating the dusts with soil. Again, the 5 % tetra- dust was as effective as the penta- dust at 20 %. At 12.5 g. of dust per 20 kg. of soil, none of the dusts retarded the rate of emergence of lettuce seedlings but the highest concentration of the tetra- series reduced the number emerging.

Foliage applications of two dusts, 5 % tetra- and 20 % penta- dusts, controlled the spread of *R. solani*.

INTRODUCTION

Brown (1935) reported the use of 'Folosan' dust in the control of fungi causing post-emergence damping-off of lettuce. This dust, containing 20 % by weight of penta-chloronitrobenzene, reduced *Botrytis* and *Rhizoctonia* attack, but checked plant growth. Smieton & Brown (1940) found that the smaller the plants and the lower the temperature when the dust was applied, the greater was the check to growth. The experiments dealt only with the 'spring' lettuce crop, of which they described the principal pathogens: *Botrytis cinerea*, *Rhizoctonia solani*, *Pythium* sp. and *Bremia lactucae*. *B. cinerea* and *R. solani* are the two most consistent causes of losses, and are also the two affected by chloronitrobenzenes.

Brown & Montgomery (1948) introduced a second dust, D.B. 905, containing 5 % by weight of 2.3.5.6 tetra-chloronitrobenzene. They observed that, on lettuce seedlings grown in frames, this dust was better than 'Folosan' as it increased survival without apparent phytotoxic action. The two dusts used in these comparisons differed not only in type and amount of active principle, but also in the

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type of filler. The results of a strict comparison between penta- and tetra-chloro-nitrobenzenes are described in the present paper. For each compound a range of dusts with differing concentrations was used and the type of filler was standardized.

MATERIALS AND METHODS

Field experiments were carried out at Slough and Harlington, in the Thames Valley, during two mild winters, 1948-9 and 1949-50. The plants were grown as detailed by Brown (1935) for the 'spring' lettuce crop. 2 g. of lettuce seed var. Trocadero

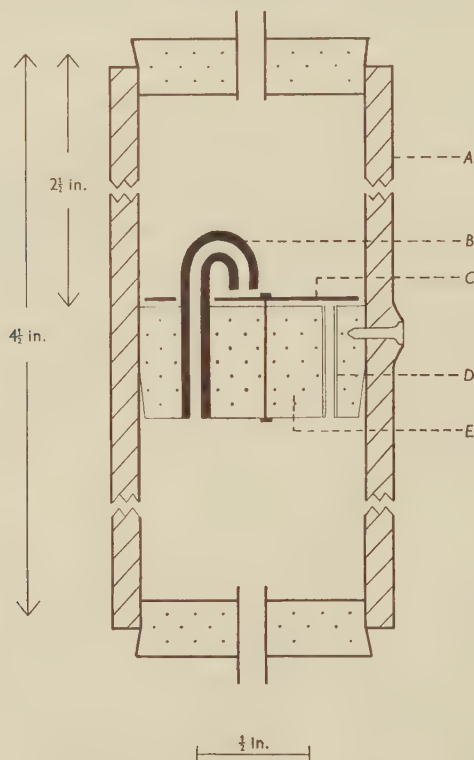


Fig. 1. Section of apparatus used in dusting. *A*, Perspex cylinder; *B*, lead tube; *C*, cardboard disk; *D*, glass tube; *E*, rubber partition.

Improved were broadcast in autumn in each cold frame, $12\frac{1}{2}$ sq.ft. in area. The frames faced south and the soil sloped from the back of each frame to the front, to improve insolation. The seedlings were transplanted into open ground in early spring and they hearted in May and June.

The soil used in five of the six experiments was almost free from *Rhizoctonia*. To control the *Botrytis* disease, tetra- and penta-chloronitrobenzenes were applied as

dusts to the foliage of seedlings growing in the frames, using a specially constructed apparatus (Fig. 1), into which air was hand-pumped at 7.5 lb. per sq.in. The apparatus is essentially a 'Perspex cylinder' (*A*) divided into an upper and a lower chamber by a rubber partition (*E*), pierced by six tubes to allow the air pumped into the lower chamber to pass into the upper chamber. Five of the tubes were straight pieces of capillary glass tubing (*D*) and did not extend beyond the partition. The sixth was a lead tube (*B*) extending into the upper chamber and bent over so that its opening in this chamber faced the centre of the partition. On the upper surface of the partition was a cardboard disk (*C*) upon which a measured quantity of dust was placed. The dust was entrained by the air flowing from the lower chamber to the upper and expelled through a glass tube ending in a fish-tail.

TABLE 1. *Details of cultivations of the series of experiments*

No. of experiment	Date of sowing	Date of first transplanting	Date of first application of dust to foliage
I	27 Oct. 48	22 Feb. 49	21 Dec. 48
II	18 Oct. 48	28 Jan. 49	2 Dec. 48
III	30 Oct. 49	20 Feb. 50	19 Dec. 49
IV	27 Oct. 49	—	8 Dec. 49
V	1 Nov. 49	24 Feb. 50	30 Dec. 49
VI	29 Oct. 49	12 Feb. 50	19 Dec. 49

After each application the frames were kept closed until the following day when the dust had settled. Undusted control frames were closed for the same period. Each frame received 9 g. of dust at each application (Smieton & Brown, 1940).

The series of dusts, with talc as the filler, was prepared with 5, 10 and 20% by weight of the active principle. For brevity, tetra-chloronitrobenzene will be referred to as TC and penta-chloronitrobenzene as PC, preceded by a number representing the percentage concentration of the fungicide, e.g. 5 TC and 20 PC.

Each treatment consisted of at least three replicate frames, except in Exp. V, in which there were only two for the dust treatments. An average of 170 seedlings were transplanted from each frame and arranged in at least four blocks. The data given are the mean values for the set of replicate frames.

Table 1 shows the details of the main operations in the six field experiments. The dates of the first dust applications are given—the final applications were made the day before transplanting. The remaining applications were made at evenly spaced time intervals.

DAMAGE CAUSED BY PC AND TC

(A) *In frames*

No symptoms appeared until 10 days after the first application of 20 TC, when the entire foliage became yellow and the edges of some leaves drooped slightly. The drooping gradually became more severe and after 2 weeks the leaves rolled downwards, becoming finally dry and papery. If the seedlings were growing very

actively when dusted, then the leaf elongating most rapidly wilted within a week and subsequently collapsed. Three weeks after the first treatment with 20TC, the seedlings were always noticeably smaller than the undusted controls. The second and third applications caused less damage; the leaves still became chlorotic but any scorching was confined to the leaf tips and entire leaves rarely collapsed.

10TC caused the same type of damage, but it developed more slowly and was less extensive. Few leaves were killed, but chlorosis and small scorch marks were general.

5TC and 20PC caused slight chlorosis but only a few plants were scorched. Usually, two doses of dust were needed to cause symptoms. The only immediate effect of 10PC and 5PC was a slight spotting of the leaves.

In addition to the damage described, the dusts accelerated the natural decay of the cotyledons and oldest leaves. These dried to a crumpled mass, which seemed unfavourable to the development of Botrytis as did all tissue damaged by the dusts.

The dusts produced a lasting check to the growth of the seedlings. Thus the size of the transplanted seedlings in Exp. I differed greatly as shown in the scheme below. The seedlings were graded by size, into five grades, *A* to *E*. Each dust treatment was replicated three times, and for the control there were eight replicates. The number of frames of each treatment allotted to a particular category is shown by the figure in brackets.

Grade <i>A</i> (smallest)	20TC	(2)				
<i>B</i>	20TC	(1), 10TC	(1)			
<i>C</i>	10TC	(2), 20PC	(3)			
<i>D</i>	5TC	(1), 10PC	(2), 5PC	(1)		
<i>E</i> (largest)	5TC	(2), 10PC	(1), 5PC	(2), control	(8),	

The persistent effects of the dusts thus paralleled the immediate effects and can be expressed

$$20TC > 10TC = 20PC > 10PC > 5TC = 5PC = \text{control}.$$

These visual conclusions were amplified by measurement of the length of the longest leaf, which was taken as a measure of the height of the seedlings, since for lettuce internodal elongation is negligible. The data presented in Table 2 are the means for sixty plants per frame, four plants being taken from each of fifteen points.

The results of Exp. IV show that the reduction in size of the seedlings following dusting closely agreed with the severity of damage recorded visually, viz.

$$20TC > 10TC = 20PC > 5TC = 10PC > 5PC > \text{control}$$

and that at similar concentrations TC reduced height more than did PC. The data for the remaining experiments confirm that 5TC was less damaging than 20PC. Differences in the fresh weight of the foliage were comparable with those in height.

The number of leaves per seedling at transplanting was not greatly affected by the treatments. The largest range was between 5.3 ± 0.1 for the controls and 6.1 ± 0.1 for treatments 10TC and 20TC.

Damage to the seedlings was still further increased if the dusts were applied more than three times (Table 2).

The 10TC, 20TC and 20PC dusts predisposed the plants to injury by severe frost.

TABLE 2. *Effect of treatments on height of seedlings in frames (cm.)*

(a) Seedlings received 3 applications of dust					
Dust treatment	Exp. III	Exp. IV	Exp. V	Exp. VI	Mean
Control	12.7	12.0	13.9	8.1	11.7
5TC	10.5	10.0	12.4	7.2	10.0
10TC	8.1	8.9	—	—	—
20TC	—	6.8	—	—	—
5PC	—	11.0	—	—	—
10PC	—	10.3	—	—	—
20PC	9.5	9.1	10.4	6.4	8.9

(b) Seedlings received 5 applications of dust		
Dust treatment	Exp. III	Exp. V
5TC	7.9	9.7
10TC	6.5	—
20TC	6.3	8.0

TABLE 3. *Experiment III. Effect of treatments on maturity index*

No. of applications of dust	Maturity index*			Days delay to maturity		
	5TC	10TC	20PC	5TC	10TC	20PC
3	11.5	13.3	14.2	2.6	4.4	5.3
5	13.0	13.9	16.3	4.1	5.0	7.4
6	13.3	—	—	4.4	—	—

Control 8.9.

First date of cutting: 15 May 1950.

* Difference between means when $n=66$ for $P=0.05=1.4$; for $P=0.01=1.9$.

(B) In the field

Although there were consistent differences in size between seedlings variously treated while in frames, the subsequent effect on their growth was variable. The maturity index, the mean date of hearting of 66.7% of the surviving crop, is calculated as for the 'average day of maturing' (Smieton & Brown, 1940). Suppose that a plants are cut at the first date of cutting, and that b, c, d , etc., are cut at successive 2 day intervals, the maturity index is given by

$$a \times 0 + b \times 2 + c \times 4 + d \times 6 \dots / a + b + c + d \dots$$

The data for Exp. III are given in Table 3.

The values show that all dust treatments delayed maturity 2.6 to 7.4 days, the order of phytotoxicity being 20PC > 10TC > 5TC. Throughout, more applications caused more delay. The maturity indices were equal for two treatments receiving the same amounts of pure TC, *i.e.* three applications of 10TC and six of 5TC.

From the eight pairs of data for the maturity indices (Table 4) and the average weight of seedling when transplanted, a negative correlation coefficient of -0.91 , (significant at $P=0.01$) was obtained. Such a clear correlation was obtained only once, but there were similar trends in Exps. V and VI (Table 4).

In Exp. I, on the other hand, there were no differences in the time to maturity which could be related to dust treatment although the seedling size varied considerably on the three transplanting dates. This result, which was probably due to plant growth being affected by severe infection with lettuce mosaic virus, is exceptional.

TABLE 4. *Effect of three applications of dust on maturity index*

Dust treatment	Exp. V		Exp. VI	
	Maturity index*	Foliage weight (g.)	Maturity index†	Foliage weight (g.)
Control	8.6	83.7	3.7	46.2
5 TC	8.6	73.3	4.5	39.4
10 TC	10.6	57.3	—	—
20 PC	9.5	59.0	6.1	33.5

* Differences are not significant.

† Difference between means when $n=30$ for $P=0.05=1.7$; for $P=0.01=2.2$.

CONTROL OF *BOTRYTIS* DISEASE

The control of *Botrytis* disease by the various treatments was estimated in the frames and later in the field.

(A) In frames

Botrytis damage did not occur until two months after sowing, when the foliage was forming a canopy above the soil. With further foliage growth the cotyledons and lowermost leaves died from lack of light and *Botrytis* could invade the stem through the bases of these moribund leaves. Lettuce leaves have no abscission mechanism.

The value of the dust treatments was assessed by the presence or absence of sporing *Botrytis* and the number of living plants in the frames immediately before transplanting.

Sporing *Botrytis* was usually found only in the control frames: in Exp. IV, however, it was also found sporing in treatments 5 PC, 10 PC and 5 TC. No allowance was made for the seedling size in applying the dust, and as these three treatments did not reduce the size of seedlings as much as did 20 PC, 10 TC and 20 TC, the lowermost leaves probably received less dust.

In all the experiments most dust treatments increased the final stand of seedlings in the frames to varying degrees. The smallest increase was in Exp. III where the treatments increased the stand by about 4% from 1255 to 1303 plants. The largest increase was in Exp. II, in which the seed was sown earlier than usual (mid-October) and the average increase was from 1147 to 1548 (35%). There were no

consistent differences between the stands given by the various dust treatments. Table 5 gives the results of examining the plants of Exp. IV at the stage when they would normally have been transplanted.

The results show that the number of seedlings in the final stand in the controls was smaller than in those in the dusted frames, except for 5PC, and that the largest increases were given by 10TC and 20TC. All the dust treatments decreased the percentage of plants with stem lesions and an increasing number of plants had stem lesions with decreasing concentration of both TC and PC. At similar concentrations, TC reduced the number of infected plants more than PC (5TC was as effective as 20PC).

TABLE 5. *Experiment IV. Disease in frames in relation to size of plant as affected by three applications of dust*

Dust treatment	Presence (P) or absence (A) of		Final stand of plants	% plants with stem lesions	Average height of plants (cm.)
	(1) Seedlings completely damped-off by <i>Botrytis</i>	(2) Sporing <i>Botrytis</i>			
Control	P	P	1149	10.18	12.0
5TC	P	A	1284	4.90	10.0
10TC	P	A	1434	1.01	8.9
20TC	A	A	1454	0.22	6.8
5PC	P	A	1111	8.10	11.0
10PC	P	A	1226	6.93	10.3
20PC	P	A	1320	5.08	9.1

The decrease in the number of plants with stem lesions may be partly due to the absence of sporing *Botrytis*, and partly to the effect of smaller plants in giving increased crop aeration, unfavourable for the fungus. There was a pronounced tendency for the percentage of stem-infected plants to decrease with seedling height (Table 5).

Although the numerical differences were small, the seedlings receiving different treatments differed considerably. Four sets of forty seedlings without obvious stem lesions were taken from each of three frames and incubated at 10° C. One frame had been dusted three times with 20TC, another with 20PC and the third was from a control series. The percentages of these plants showing *Botrytis* lesions a week later were as follows: control, 64.2; 20PC, 27.5; 20TC, 2.0.

(B) In the field

In Exp. I batches of seedlings treated with the 5, 10 and 20% dusts of PC and TC were transplanted on three occasions, at intervals of 10 days. They were dusted immediately before lifting on the first two occasions but not on the third. Table 6 shows that differences in the frames were negligible but that there were large differences in survival in the field. For the first date of transplanting, all the dusts improved survival. The smallest increases were given by 10PC and 5PC; the

largest by 20TC. The treatments arranged in order of increasing percentage survival are:

$$20TC > 10TC > 5TC > 20PC > 10PC = 5PC > \text{control}.$$

At similar concentrations TC was superior to PC and with each increase in concentration of TC but not of PC the percentage survival progressively increased.

The percentage survival of the control plants in the successive transplantings declined rapidly; a delay of three weeks reduced survival from 41.4% to 4.7%. In the second transplanting, the survival in the 5TC, 5PC, 10PC and 20PC treatments was also reduced; the high level of survival given by 10TC and 20TC was maintained.

TABLE 6. *Experiment I. Survival data in relation to three dust treatments and date of transplanting*

Dust treatment	Final stand in frames	% survival in field on 10 May 1949. Crop transplanted on		
		22 Feb.*	3 Mar.†	15 Mar.
Control	1063	41.4	19.8	4.7
5TC	1137	74.0	43.0	—
10TC	1387	81.3	73.4	—
20TC	1330	89.7	87.0	76.1
5PC	1352	57.6	42.5	—
10PC	1291	55.5	31.9	—
20PC	1204	61.6	30.5	28.9

Differences between means for $P=0.05$,* 12.9 ($n=75$),† 17.1 ($n=28$).

In this experiment, which was exceptional, no dust treatments produced any measurable check to the plants. Other experiments have shown, however, that of the six dusts 20TC caused most damage. Therefore this treatment was omitted in the remaining experiments as were 5PC and 10PC treatments. The results for Exps. II, III and V are summarized in Table 7.

As before, all the dust treatments increased survival. In Exp. III, 10TC induced higher survival than 5TC or 20PC. Including the data for Exp. I (Table 6) 20PC > 5TC in two out of the four experiments, while 5TC > 20PC in the other two. 5TC and 20PC gave 54.2 and 51.4% survival respectively (27.1% in control).

5TC and 20PC applied four or more times gave higher survivals, in two out of three experiments, than when applied thrice. Table 7 shows that in Exp. II, the fourth application of 5TC significantly improved survival and in Exp. V, five applications of 5TC and 20PC gave increases. Although the survival in Exp. III after three applications of either 20PC or 5TC was not 100%, the additional applications had no further effect. This may be associated with lack of initial infection in the frames.

TABLE 7. *Survival data in relation to number of applications of dust*

	Exp. II		Exp. III		Exp. V	
	Final stand in frames	% survival in field	Final stand in frames	% survival in field	Final stand in frames	% survival in field
Control	1147	13.0	1255	19.5	1131	41.8
(a) Three applications of dust:						
5 TC	1597	26.6	1373	65.1	1505	62.1
10 TC	—	—	1214	84.2	—	—
20 PC	1565	31.9	1308	57.9	1302	75.2
(b) Four applications of dust:						
5 TC	1481	47.7	—	—	—	—
(c) Five applications of dust:						
5 TC	—	—	1324	67.6	1429	87.1
10 TC	—	—	1343	78.9	—	—
20 PC	—	—	1216*	59.8	1152*	82.2
(d) Six applications of dust:						
5 TC	—	—	1342	70.6	—	—
Significant difference between means for $P=0.01$		12.0 ($n=33$)		20.3 ($n=66$)		15.3 ($n=35$)

* The decreased final stand of seedlings in the frames treated 5 times with 20 PC as compared with those treated only 3 times, was due to an increased predisposition of the seedlings to frost damage, which resulted in a number of them being killed.

THE CONTROL OF *RHIZOCTONIA* ATTACK

The soils used in the experiments already described were almost free from *Rhizoctonia*. In preliminary pot experiments, PC and TC dusts were incorporated with the top 2 in. of soil at the rate of 12.5 g. of dust per 20 kg. of unsterilized soil. At a higher dose, all the TC dusts retarded emergence and reduced the final number of seedlings whereas the PC dusts had no effect. Thirty lettuce seeds per pot were sown within the layer of dust-treated soil in three concentric circles of 5, 10 and 15 seeds respectively. A disk of *Rhizoctonia* inoculum on potato-dextrose agar $\frac{1}{2}$ in. in diameter was placed at the centre of the dust-treated layer. For each treatment there were four replicate pots.

The 5, 10 and 20% dusts of both PC and TC were tested in one experiment done in an unheated greenhouse during the winter. The seedlings in all treatments emerged at the same rate. Seven days after the start differing amounts of fungal growth were visible. The hyphae penetrated the covering soil and formed a superficial mycelium in all treatments but its density was reduced by 10 TC, 20 TC and 20 PC. Concentric growth of the mycelium soon ceased in the dust treatments but continued in the untreated pots until the inner ring of seedlings had been attacked. The percentage survivals after 11 weeks are given in Table 8 which shows that all the dusts except 5 PC significantly improved survival. The percentage survival increased with each increase in the concentration of PC, in contrast to the results obtained against *Botrytis*. Treatment with 5 TC produced the same survival rate as with 20 PC, 10 TC and 20 TC.

A field experiment, Exp. VI, was made with soil from a lettuce seed-bed which was heavily contaminated with *Rhizoctonia*. In addition to dusting foliage, the four dusts, found equal in inhibiting *Rhizoctonia*, viz. 5TC, 10TC, 20TC and 20PC, were incorporated in the top 2 in. of soil again at the rate of 12.5 g. per 20 kg. before the soil was distributed in the frames.

Emergence was assessed by the time taken for a hundred seedlings per frame to emerge and spread their cotyledons. This stage was reached in all frames 10–12 days after sowing and no treatment differences were observed. The total emergence in each frame was estimated 3 weeks after sowing by counting the seedlings within fifteen quadrats (each 16 sq.in.) per frame. Table 9 shows that 20TC was the only soil amendment to reduce emergence.

TABLE 8. *Percentage survival in soils, with which dust had been incorporated to control Rhizoctonia attack*

Inoculated with <i>Rhizoctonia</i>							Uninoculated
Control	5PC	10PC	20PC	5TC	10TC	20TC	Control
28.6	34.5	65.9	82.5	79.8	81.5	73.5	92.9

Difference between means for $P=0.05=7.3$ when $n=30$.

TABLE 9. *Exp. VI. Effect of incorporation of dusts in soil upon the emergence of lettuce seedlings, and the incidence of Rhizoctonia attack*

Soil treatment	Emergence of seedlings per 15 quadrats	Number of centres of <i>Rhizoctonia</i> attack
	21 Nov. 1949	12 Dec. 1949
Control	334 ± 9.2	13.5 ± 1.0
5TC	338 ± 5.5	9.6 ± 2.2
10TC	346 ± 4.7	6.3 ± 1.5
20TC	297 ± 2.6	9.0 ± 2.1
20PC	334 ± 12.5	4.7 ± 0.6

When transplanted the seedlings in all treatments had the same number of leaves but differed in height. With either 5TC or 20PC as the foliage dust, the soil amendments 5TC, 10TC, 20TC and 20PC only slightly reduced height, as shown in Table 10 which also gives the maturity indices. This table shows that the greatest effects on height and maturity index were caused by the foliage treatments, 20PC delaying maturity more than 5TC. The addition of the dusts to the soil consistently produced a further delay, but this was not significant. Frost damage was not affected by the soil treatments.

Damped-off seedlings occurred simultaneously in all treatments and specimens were first observed 3 weeks after sowing. The centres of infection in each frame were recorded on 12 December, 5 weeks after sowing, before the first foliage dusting. Table 9 shows that all the soil treatments reduced the number of outbreaks of damping-off, and that 20PC was the most inhibitory.

Rhizoctonia in the treated soils did not spread appreciably from the initial centres of infection until the end of January. It was at this time that *Rhizoctonia* became active in another experiment in which 20PC and 5TC were being used as soil amendments. Virulent attacks occurred in November and late January in the untreated soils, regardless of whether or not the foliage was later dusted.

Table 10 shows that the smallest stand (672 plants) was obtained in the control frames, and the largest, (1321 on an average), were in those receiving both soil and foliage treatments; those in which only the foliage was dusted were intermediate with 986 plants (20PC gave a greater increase than 5TC). The four soil amendments were at this stage equally effective in controlling *Rhizoctonia*, the differences noted earlier (Table 9) now being eliminated.

TABLE 10. *Exp. VI. Effect of incorporation of dusts in soil upon the % survival and maturity index*

Soil treatment	Foliage treatment	Final stand in frames	Aver. height of plants (cm.)	% survival	Maturity index*
Nil	Nil	672	8.1	86.0	3.7
Nil	5TC	947	7.2	89.0	4.5
Nil	20PC	1025	6.4	86.2	6.1
5TC	5TC	1338	7.2	88.9	5.6
5TC	20PC	1344	6.3	90.2	6.3
10TC	5TC	1313	6.8	88.4	5.1
20TC	5TC	1283	7.2	90.6	5.0
20PC	5TC	1283	7.1	87.2	5.1
20PC	20PC	1364	6.5	86.5	7.4

* Difference between means when $n=30$ for $P=0.05=1.7$.

There were no differences in the percentage survivals in the field although the amount of disease in the frames had differed greatly between treatments. This result contrasts with that in Exp. III which was carried out in an adjacent block of frames, and in which severe losses in the controls occurred in the field (20% survival), following slight attacks in the frames. Before transplanting, the seedlings in the control frames of Exp. III were more overcrowded than those in Exp. VI, (1255 seedlings per frame against 672), and each seedling was larger (12.7 cm. high against 8.1 cm.) Under these conditions the cotyledons were prematurely killed by lack of light and the seedlings consequently were vulnerable to *Botrytis*. In the control series in Exp. VI which had been severely attacked by *Rhizoctonia*, the cotyledons were healthy when transplanted.

RECOMMENDATIONS

When considering the use of a fungicide, it is necessary to take into account its phytotoxicity. The TC dusts reduced *Botrytis* disease more effectively, but also caused more damage to lettuce than the PC dusts at similar concentrations. Although 20TC gave most reduction in *Botrytis* disease, it would be impractical to use

because it causes too much damage. On the other hand, 5PC and 10PC are insufficiently fungicidal. Of the remaining dusts 10TC gave better control of *Botrytis* than either 5TC or 20PC, while 5TC caused less damage than 10TC or 20PC. Consequently both 5TC and 10TC are preferable to 20PC. Dusting with 5TC, in contrast to that with 10TC, does not predispose plants to frost damage, and therefore would not too greatly endanger the growth of lettuces.

Four dusts when mixed with soil, i.e. 5TC, 10TC, 20TC and 20PC controlled equally the spread of *Rhizoctonia*. As 20PC, in contrast to the TC dusts, had no effect on the emergence of lettuce seedlings at unusually high doses, it may be safely used for the control of *Rhizoctonia*.

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OBSERVATIONS ON APPLE CANKER

IV. THE INFECTION OF LEAF SCARS

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(With Plate 20 and 3 Text-figures)

Field observations on leaf-scar canker of apple confirm its importance in the south-west of England: on a heavily infected tree more than 50 % of the current year's growth may be damaged. Leaf scars may become infected by spores of *Nectria galligena* which are sucked into the tracheids of a freshly exposed scar where they develop in a relatively protected environment. These freshly exposed scars become much less susceptible to infection after approximately one hour.

Infections established in leaf scars may develop immediately into cankers or they may be confined by a suberized or gum barrier. Mycelium from some of these confined lesions may spread into the surrounding healthy tissue through growth cracks when the buds develop. The lesions extend throughout the winter and spring; usually the scars earliest inoculated are the first to show cankers. It seems possible that infections of this type might be controlled by eradican sprays applied just before leaf-fall.

INTRODUCTION

This contribution forms part of a series on the biology of the canker disease of apple, caused by *Nectria galligena* Bres., which is being studied at Long Ashton Research Station; the earlier papers have been contributed by Marsh (1939), Munson (1939) and Crowdy (1949).

Apple canker is especially prevalent in the west of England, where one of its most serious manifestations is the infection of leaf scars. The relationship between leaf scars and the cankers associated with buds on young stems was first established by Wiltshire (1921). Grubb (1921) in observations on the incidence of canker at East Malling also recorded a high percentage of leaf-scar infection.

Wiltshire (1921) noted that there were two main periods when infection developed; in early autumn, when the cankers may appear on the lowest leaf scars before the upper leaves have been shed, and also in spring; he suggested that infection took place in autumn through cracks which follow the drying-out of the scars, and in spring through cracks in the bark following the swelling of the buds. In addition to these peak periods it appeared that some infection could occur throughout the rest of the year. The dates of the peak periods appeared to differ with different varieties, and Wiltshire noted a tendency for varieties which held their

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leaves late into the autumn to be less susceptible than those which shed their leaves early. He found leaf-scar infection to be dependent to some extent on weather and noted that it was rare on the 1919 wood but heavy on the 1920 wood following excessive rain in the early autumn of 1920.

Zeller (1926) made a study of leaf-scar infections on pomaceous fruit in the United States of America. Infection on pear was found to be heavy, though varieties which held their leaves till late in the season might escape. In Oregon leaf-scar infection occurred mainly during the autumn though it might occur during the milder periods of the winter, and rather more frequently during the spring. Zeller inoculated leaf scars with conidia and ascospores; when inoculation followed immediately on defoliation there was, in general, a high percentage of infection, but little infection occurred when a week elapsed between leaf-fall and inoculation: on the whole ascospores were found to be more effective than conidia in these trials. He suggested that capillary suction (Brooks & Moore, 1923) might play some part in the establishment of infection. Zeller also found that he could obtain a successful control of infection with a Bordeaux spray applied either immediately before inoculation, or a week after; unfortunately a week was the longest interval tested so it does not represent the longest allowable interval for successful control.

Further artificial inoculations were carried out by Marsh (1939). In these experiments fragments of agar bearing mycelium of *N. galligena* were placed on leaf scars at different times of the year and covered with a crepe bandage. These experiments showed that infections resulted only from inoculations made in the autumn and the spring, and that in 1937 they occurred much more readily in the spring. Marsh also demonstrated that Bordeaux mixture was much more toxic to the spores of the fungus than lime sulphur under laboratory conditions, and showed that the application of a Bordeaux oil spray prior to inoculation markedly reduced infection.

In addition to these attempts to control artificially induced infections there are some observations on the effect of sprays on the incidence of the disease in the field. Wiltshire & Spinks (1921) claimed a substantial reduction in infection following a winter spray with copper stearate, but Wiltshire (1923) was unable to repeat this result. Grubb (1921) reported that a substantial reduction in leaf-scar infection was produced by a programme of fungicide sprays during the summer preceding leaf fall. Grubb also observed that leaf-scar infection occurred mainly on trees which were already infected with canker, and that a tree which was free of canker was unlikely to suffer from leaf-scar infection.

It is common experience that an intensive summer spraying programme with sulphur fungicides does not provide a satisfactory control of canker disease, even though it may, by indirect means, produce some reduction in intensity. Neither does it appear possible to effect a control by the normal protectant spraying in the winter. In these circumstances it was felt that an investigation into the formation and development of leaf-scar cankers might provide data which would explain some of the observations noted above and also indicate a method of control.

OCCURRENCE OF LEAF-SCAR CANKERS IN THE FIELD

As has been noted above, leaf-scar cankers have been recorded as causing serious damage in the field but no quantitative estimates of the damage have been made. The distribution of the disease may be estimated either as the average number of infected leaf scars per shoot or the proportion of shoots which carry infection; of these the latter probably gives the better estimate of the damage caused to the tree, since a single infection frequently girdles and kills a shoot in the season following infection and if the shoot is diseased it will probably be cut out at the earliest opportunity. The relation between these two estimates of disease intensity will be considered in more detail later.

In estimating the percentage of shoots infected, a random selection of shoots of the previous season's growth were examined and the number which carried cankers recorded. It has been found in practice that the distribution of the disease in an orchard is extremely erratic and varies profoundly from tree to tree and from season to season. Counts, do, however, illustrate the damage which can be caused.

TABLE 1. *Percentage of shoots infected with leaf-scar canker*

Variety	No. trees examined	% shoots cankered/tree	
		Mean	Maximum
Worcester Pearmain	10	20	55
Cox's Orange Pippin	10	13	45
Court Royal	3	43	46
Ashton Red	1	—	74

Table 1 assembles a series of counts made in a well-managed plantation at Long Ashton illustrating how serious this disease may be in the west of England.

In estimating the number of cankers per shoot, approximately 100 shoots were examined at random on a tree and classified according to the number of cankers occurring on each; if infection was at random the number of shoots in each class should follow a Poisson distribution. Text-fig. 1 illustrates the frequency distribution of cankers on a heavily infected tree and also the expected Poisson distributions. The recorded and calculated frequencies were compared by the χ^2 test and the results show that the agreement is close in each case.

When the rate of infection is high, the distribution of leaf-scar cankers is at random and there is, therefore, a precise relationship between the mean number of cankers and the number of shoots carrying infection. With low rates of infection the distribution of infection ceases to be at random and this relationship disappears. The calculations show that there is a tendency for the infections to aggregate within the tree.

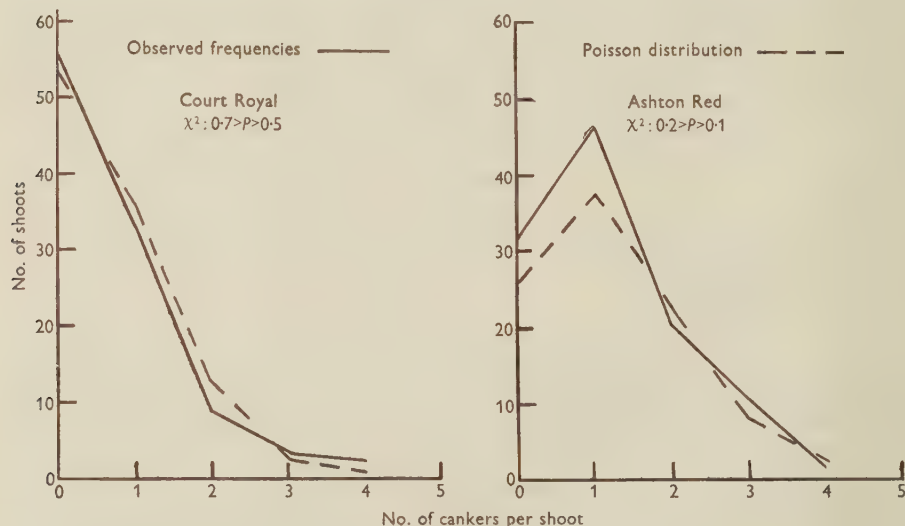
FORMATION AND DEVELOPMENT OF LEAF-SCAR INFECTIONS

Mention has already been made of Zeller's observation that the inoculation of leaf scars with spores was much more effective if carried out immediately after leaf-fall and his suggestion that this might be due to some capillary effect. It was also noted

early in the present series of trials that infection drops were sucked readily into the freshly made leaf scars. The following data were collected to investigate the entrance of spores into the leaf-scar traces and to find whether infections established in this way were of economic importance.

It should be noted that there is some evidence (Byrde, Crowdy & Roach, 1952) that this capillary suction effect accounts for only part of the infection recorded.

Munson (1939) has studied spore formation on apple cankers and shown that when leaf-fall in most varieties occurs the production of conidia is at its peak or on the decline and ascospore discharge just starting. As conidia are thus the predominant source of infection in early autumn, conidial suspensions in water have been used exclusively in the inoculation trials. In early experiments the conidia



Text-fig. 1. Distribution of leaf-scar cankers on stems of heavily infected trees.

were derived from pure cultures but as the pathogenicity of the fungus was apparently reduced by repeated transfers the conidia for later trials were taken directly from cankers collected in the field and incubated, if necessary, in a saturated atmosphere.

Preliminary observations indicated that apple leaves ready to fall were very weakly attached to the tree and that the final detachment was mechanical and usually caused by wind. In the experimental infection of fresh leaf scars the inoculations were usually made at about the time of natural leaf-fall: the leaves were removed by hand and a drop of aqueous spore suspension applied with a pipette to the scar thus made.

Microscopic examination of the freshly exposed leaf scars showed that no pro-

protective layer was formed prior to leaf-fall, so there was no mechanical barrier to bodies which were small enough to enter the tracheids. Conidia and ascospores have been found to penetrate readily into the leaf-scar tracheids of all the varieties examined, namely Bramley's Seedling, Beauty of Bath, Cox's Orange Pippin, Laxton's Superb, Lord Lambourne, Rival and Worcester Pearmain. A typical penetration is illustrated in Plate 20, fig. 1. The spores are unable to pass the cell wall between two tracheids but many spores penetrate sufficiently deeply to be protected from desiccation. The conidia thus deposited in the tracheids germinate readily and even in the beginning of December germinating conidia have been seen in the tracheids 24 hours after inoculation in the open. After germination there is a steady growth of the mycelium which, after 4 weeks, is clearly developed in the tracheids near the leaf scar, but has not spread into the surrounding tissue. This stage is illustrated in Plate 20, fig. 2, which shows the spores and mycelium of a naturally infected leaf scar in Bramley's Seedling sectioned in mid-January.

TABLE 2. *The type of infection established after leaf-scar inoculation*

Variety	% lesions		% total infection	
	Spreading	Confined	Inoculated	Controls
Beauty of Bath (a)	9	70	78	0
Beauty of Bath (b)	11	28	39	7
Cox	32	20	52	3
Worcester	6	2	8	3
Laxton's Superb	59	6	65	0
Bramley (a)	0	35	35	0
Bramley (b)	2	25	26	12

The subsequent development of the lesions seems to depend entirely on the reaction of the host to infection. A proportion of these incipient infections will develop into typical leaf-scar cankers in all the varieties listed above, though cankers are produced only rarely on Bramley's Seedling. When cankers are not developed a protective suberized or gum layer is formed ahead of the incipient infection and this appears effective in preventing the further development of the lesion (Plate 20, fig. 4). The fungus, however, remains alive in these confined lesions for at least 4-5 months after inoculation. Confined infections can be detected in the field as localized discolorations in the vascular tissue of the leaf scar when a transverse cut is made immediately below its surface.

Some numerical observations have been made on the incidence of these infections on inoculated shoots. The shoots were inoculated in the usual way about the time of leaf-fall and were collected the following spring at bud-burst. The leaf scars were then classified either as uninfected, or as confined lesions, restricted to the region of the vascular tissue of the leaf scar, or as spreading lesions in which the surrounding tissue was infected. The results of these observations are shown in Table 2 which also shows clearly that leaf-scar cankers can be initiated with this type of inoculation.

In both Bramley (*a*) and Beauty of Bath (*a*) the traces in the scars were either cultured or examined microscopically and this more detailed examination may partly account for the higher numbers of confined infections recorded. The total rate of infection covers so much variability in the behaviour of the different varieties at different times that it is not possible to reach definite conclusions on varietal differences, though it is, perhaps, significant that the varieties commonly considered canker-susceptible show a higher proportion of spreading to confined lesions.

The period of time during which the fresh leaf scar will continue to suck in liquids is of importance when considering the susceptibility of the scar to the establishment of this type of infection. The effectiveness of the suction was examined by placing drops of dilute aqueous safranin on the freshly made scars. During the first 5 minutes after the removal of the leaf the drop disappeared immediately and the dye could be traced in the xylem for about 3.4 in.; after 15 min. the uptake was slower but the depth of penetration was roughly the same.

TABLE 3. *The effect of the lapse of time between defoliation and inoculation on the susceptibility of leaf scars*

Time after defoliation (hr.)	% of inoculated scars forming cankers	
	Worcester Pearmain	Beauty of Bath
0	24	53
0.5	41	20
1.0	8	27
2.5	8	32
6	14	—
24	11	—
48	0	20
uninoculated	7	13

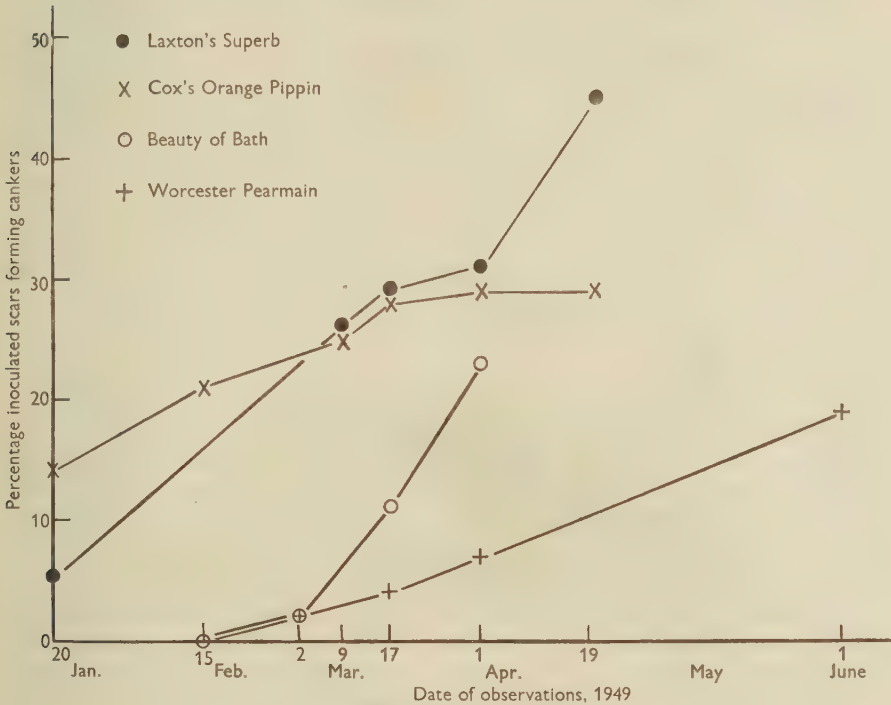
After 1 hr. the drop disappeared very slowly but there was still fair penetration, of the order of 1.5 to 2 in., and active suction had virtually disappeared after 2 or 3 hr. It would therefore seem that there is a very short period of highly active suction following immediately on the removal of the leaf. This is probably the result of breaking the transpiration stream flowing to the leaf. The initial period seems to be followed by a much longer period, of 2 or 3 hr. when there is appreciable suction but in which the depth of penetration and the rate of water uptake decreases rapidly with time. After this, active suction appears to cease.

Table 3 shows the effect of inoculating leaf scars in Worcester Pearmain and Beauty of Bath at various times.

In this experiment the occurrence of cankers is very much as would have been expected if active suction was an important factor in the establishment of the infection.

Trials similar to those described above have been carried out on cut shoots in the laboratory and while the results do not warrant a detailed consideration they

do raise certain points of general interest. Shoots bearing leaves were collected in the field, placed in pots containing damp sand and defoliated. The leaf scars on these shoots were either inoculated immediately on defoliation or held for varying periods prior to inoculation in a saturated atmosphere. When inoculation was delayed the shoots were also held in a saturated atmosphere for 3 days after inoculation. After treatment the shoots were all stored in a warm greenhouse until



Text-fig. 2. Time of development of cankers on inoculated shoots.

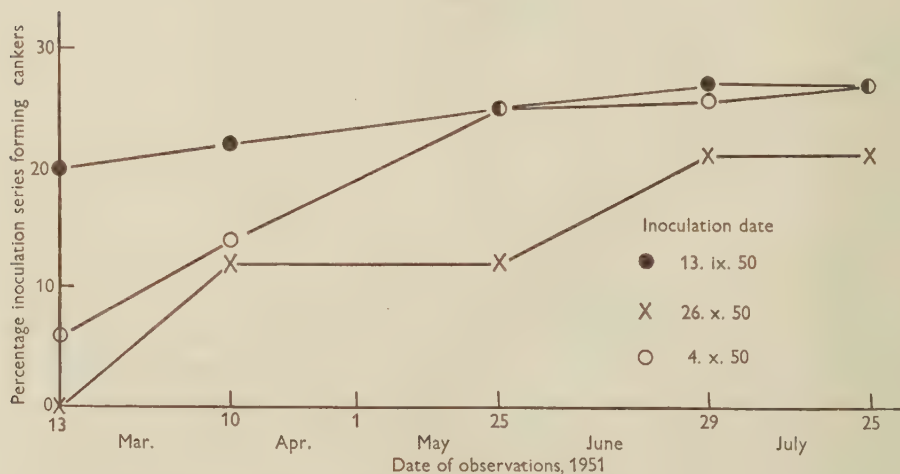
cankers developed. In these conditions Bramley's Seedling which had been inoculated immediately after the leaves had been removed showed between 90 and 100% infection. The infection rate remained high and relatively constant between 60 and 70% for about 24 hours following defoliation after which it dropped to zero. These observations show that in the conditions of this experiment, between 24 and 48 hours elapsed before an effective barrier to infection was formed. It seemed that prior to the formation of the barrier the scar was susceptible to infection by spores lodged on its surface provided conditions were suitable for their development. It is also interesting to note that the field resistance of Bramley's Seedling

apparently depends on the ability of shoots to form an adequate barrier to the spread of incipient leaf-scar infections.

There is considerable variation in the time which it takes shoots inoculated in the field to develop visible cankers. The first external sign of a canker is a dark sunken area in the stem near the leaf scar. This infected area does not take on

TABLE 4. *Cankers recorded on 25 July 1951 resulting from the inoculation of leaf scars at different dates (20 scars inoculated on each tree at each date)*

Tree	Date of inoculation, 1950						Total cankers formed	
	September			October			Inoculated shoots	Uninoculated controls
	13	21	29	4	19	26		
A	9	2	0	10	0	2	23	2
B	1	0	0	6	0	5	12	0
C	3	0	0	0	0	4	7	1
D	2	1	0	1	0	0	4	0
E	12	3	3	10	3	13	44	15
Total cankers	27	6	3	27	3	24	90	18



Text-fig. 3. Effect of time of inoculation on development of cankers on Laxton's Superb.

a characteristic cankered appearance unless the shoot is growing, when the dead area is left behind and tends to split. For this reason most cankers become obvious with the onset of vegetative growth in the spring. Characteristic cankers which are seen on leaf scars early in the winter have usually resulted from infection formed before the cessation of stem growth. Text-figure 2 summarizes observations on the occurrence of the earliest signs of canker formation following the inoculation of leaf scars on a number of varieties.

The time at which the cankers become visible appears to be affected by the date of infection. To examine this effect twenty freshly made leaf scars were inoculated weekly on each of five adjacent trees of Laxton's Superb from the middle of September until leaf-fall was practically complete. The same spore suspension was used for all the inoculations on any one date and the spore suspension was made up freshly on each date with conidia from active cankers. The cankers resulting were counted on 25 July 1951 and are recorded in Table 4 and in Text-fig. 3. It is apparent from this figure that the earliest symptoms may be expected for the scars inoculated earliest in the autumn.

The wide variation shown in Table 4 illustrates the difficulty of using data obtained in this way to make precise comparisons of varietal behaviour. There appear to be consistent differences in the susceptibility of different trees of the same variety in the same area. This difference is reflected in the rates of natural infection as well as in the susceptibility of the trees to infection following inoculation. Superimposed on this tree-to-tree difference there is a marked difference in the success of inoculations on different dates other than that related to the stage of development of the tree at the time of inoculation. It is not possible to suggest any reason for the latter variation but it does not appear to be due to any difference in weather sufficiently large to be noted by routine meteorological recording.

DISCUSSION

Leaf-scar infections of the type which have been described above raise a number of points of interest in the ecology of apple canker. The active suction of the spores into the xylem has, so far as the author is aware, only been recorded in two other diseases, silver leaf of plum, in which the spores of *Stereum purpureum* were seen to be sucked into the xylem of freshly cut twigs (Brooks & Moore, 1923) and bacterial canker of cherry, in which the avenue of infection was the leaf scar (Crosse, 1951). This method of entry is likely to be of particular importance when the organism is distributed in meteoric water. When the spore has been taken up by capillary suction the critical early stages of the development of the disease are completed in a protected environment within the host and this should considerably enhance the chances of infection.

Munson (1939) has shown that the predominant spore of *Nectria galligena* at the time of leaf-fall is the conidium and he has further noted that the release of conidia is dependent on the presence of water and that their distribution appears to be in water drops. The tendency of the infections in the trees to be aggregated, which was noted in the field observations, is more compatible with dissemination by rain-splashing than by wind. Where an adequate supply of spores is present the rate of infection will thus be mainly influenced by the presence of water at the time of leaf-fall. In this connexion it is noteworthy that the south-west of England, where leaf-scar canker is very serious, is situated in a belt of prevailing westerly winds and that strong winds bearing rain are a feature of the climate in autumn. These

winds provide simultaneously not only for the removal of the leaves but also for water for the distribution of the conidia. Thus the conditions for the development of leaf-scar cankers are ideal.

The later development of these cankers also presents problems. The condition of the host may determine the speed and efficiency with which a protective barrier is formed round an incipient lesion. An extreme illustration of this effect is seen by contrasting the behaviour of shoots of Bramley's Seedling in pots of sand with those attached to the tree. The variability of the trees of Laxton's Superb shown in Table 4 may be a further illustration of this effect. In addition to the variability of the host there are indications, also illustrated in Table 4, that environmental conditions at the time of inoculation affect the pathogenicity of the fungus. The variations due to these causes outweigh the differences between most of the varieties in the trials recorded above and make it impossible to draw up a scale of varietal susceptibility.

The infections established with conidia sucked into the tracheids of the leaf scar may develop directly into cankers or they may be confined within a protective barrier. Wiltshire (1921) noticed that cracks were formed in the leaf-scar tissue when the buds developed in the spring and Marsh (1939) showed that at this time of year leaf scars could be infected with mycelium applied to their surface. This suggests that the growth cracks rupture mechanically the protective layer thus rendering the leaf scars susceptible to infection. The confined infections have been shown to contain live mycelium actually within the tissues of the scar and it seems probable that this mycelium is also capable of growing back into the healthy tissue through growth cracks.

It has been shown on certain varieties that infections can continue to develop into July (Text-fig. 2) and that there is an apparent break in the smooth development of the cankers in the last inoculated series shown in Text-fig. 3. It is difficult to explain these effects on the hypothesis that the cankers have been developing gradually since the previous autumn, but this result might be expected if one assumes that the later cankers were formed from the spread of a confined lesion into growth cracks occurring during the spring. This may well be the fate of a proportion of the confined lesions.

The economics of disease control is inevitably bound up with the loss caused by the disease. The figures quoted in Table 1 are sufficient to show that apple canker can cause a serious loss of young growth and this over a period will affect tree shape and vigour. The loss of young growth, however, may not be the only damage caused by this phase of the disease. Crowdy (1949) described a form of latent stem canker which occurred on healing wounds. In these cankers the fungus appeared to have spread from the cut surface of the pruned shoot. It seemed likely that many of the shoot bases involved in this type of canker were originally infected through their leaf scars. These cankers often appear on large branches and may cause a disproportionate amount of damage.

CONTROL

The leaf-scar infection presents many features which complicate the formulation of control measures. The infections are established very soon after the leaves have fallen and the spores are lodged immediately in a relatively protected environment. The period of leaf-fall may extend over at least two months and during the whole period fresh wounds will be exposed. In these circumstances application of conventional protective fungicides is unlikely to give effective control. Experiments with eradicant fungicides have, however, shown that phenyl mercury chloride can be applied safely prior to leaf-fall at concentrations which will prevent the sporulation of cankers in the field for considerable periods (Byrde *et al.* 1952). It would therefore seem possible to suppress sporulation during the critical period for infection, and eradicant sprays having this action might help materially in controlling this type of canker. How far such sprays will prove economic is an entirely different question. In an established orchard the patchy distribution of the disease might well make a general spray unnecessary and effective control could probably be secured by an intensive removal of cankers on the worst affected trees and the removal of cankered shoots during normal pruning. On the other hand in the nursery or in new plantings where the shaping of trees is of paramount importance it is necessary to avoid cankers on the shoots which will form the framework of the tree. In these cases a general prophylactic treatment may well prove of value.

The author would like to acknowledge with gratitude the interest shown by Mr R. W. Marsh during the course of the work, and the assistance rendered by Miss M. Marlow and Miss P. Tozer in the field work. The author is also indebted to Prof. Harris for permission to use the photographic equipment of the Zoology Department of Bristol University and to Mr Wing of this Department for taking the photographs.

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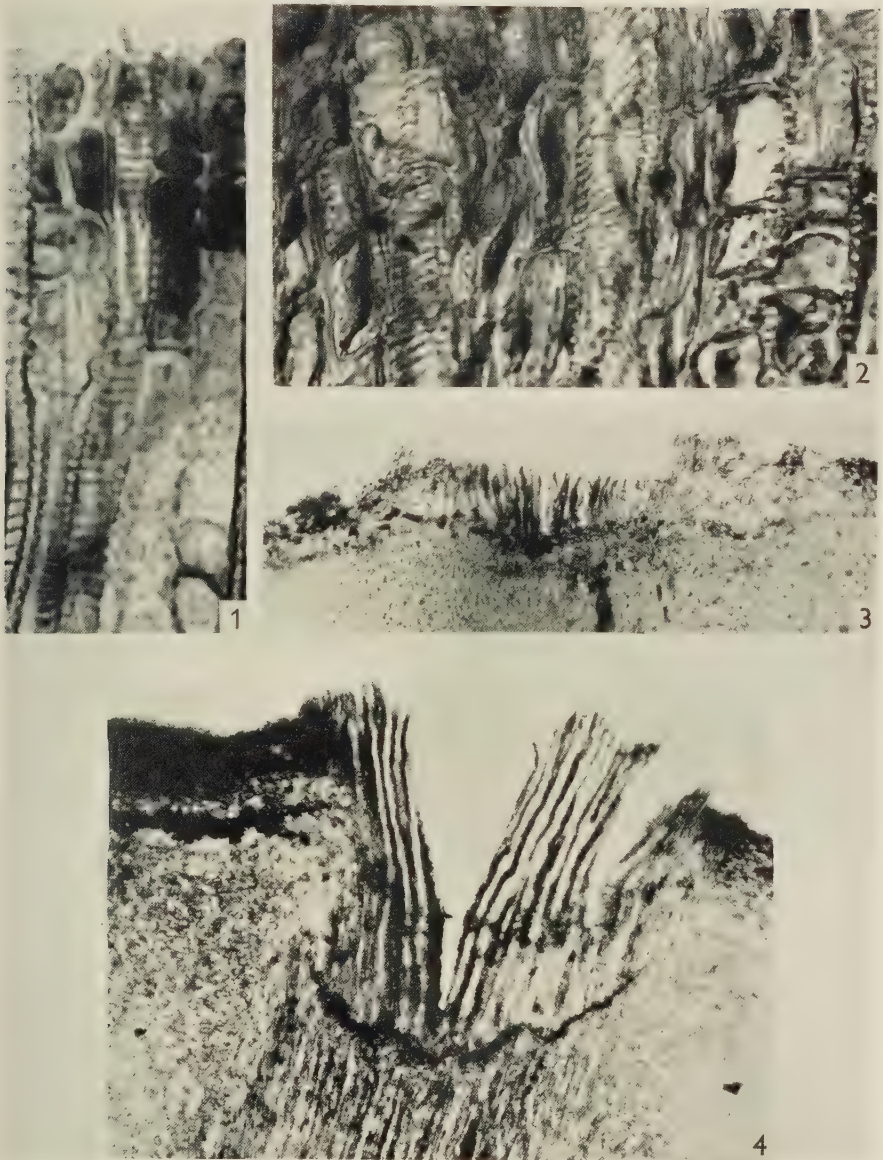
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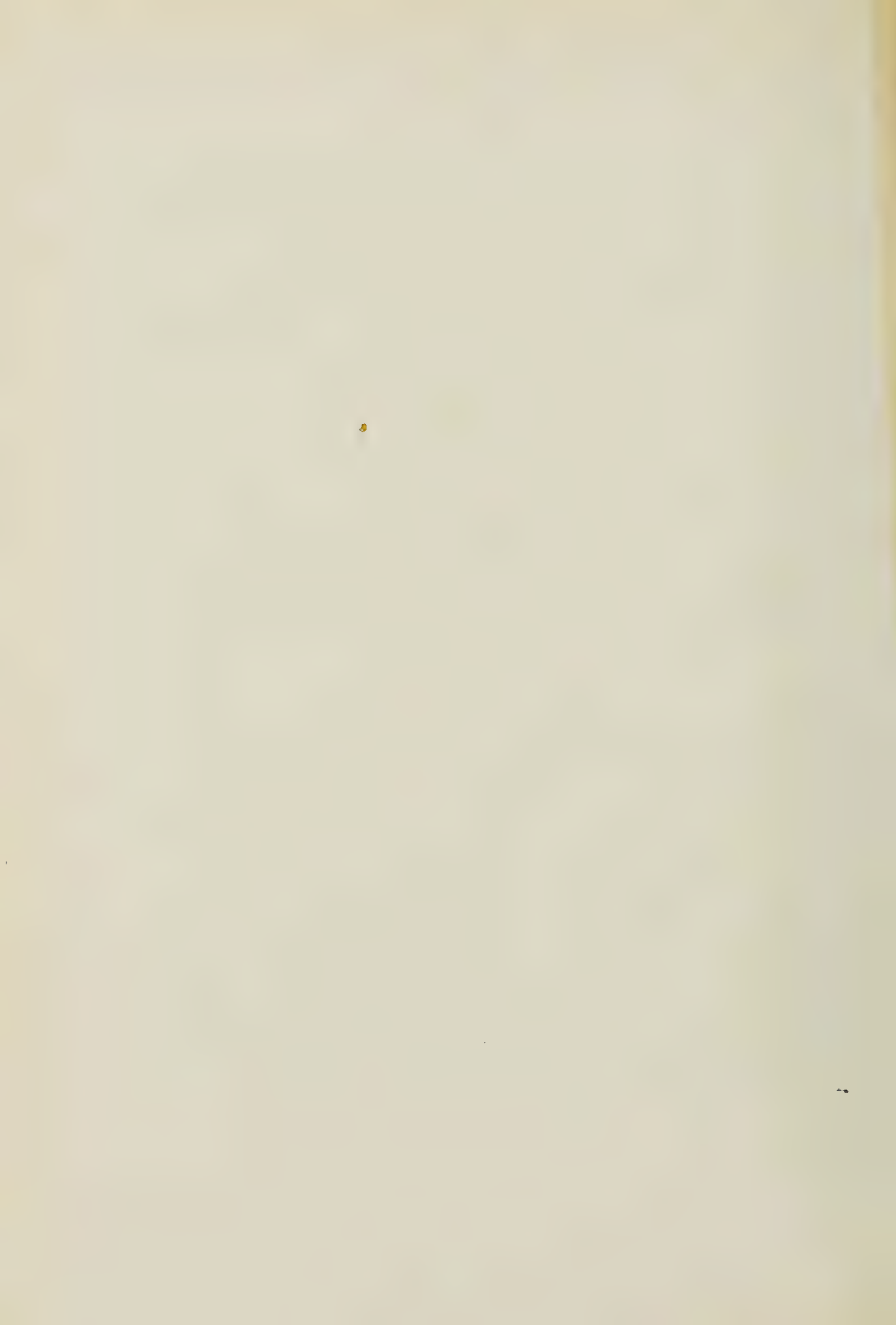
EXPLANATION OF PLATE 20

- Fig. 1. Longitudinal section of leaf scar: conidia sucked into the tracheids at the surface of the scar ($\times 600$).
- Fig. 2. Longitudinal section of leaf scar: germinating conidium and mycelium in naturally occurring infection ($\times 600$).
- Fig. 3. Longitudinal section of uninfected leaf scar ($\times 50$).
- Fig. 4. Longitudinal section of leaf scar: confined infection ($\times 50$).

(Received 11 February 1952)



CROWDY—*Observations on apple canker*



OBSERVATIONS ON APPLE CANKER

V. ERADICANT SPRAYING AND CANKER CONTROL

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AND

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Sodium pentachlorophenate and phenyl mercury chloride have been tested as eradican sprays against apple canker. Trials with inoculated shoots showed that neither of these sprays would affect an established leaf-scar canker. Both chemicals checked sporulation of *Nectria galligena* on cankers but phenyl mercury chloride was the more effective and reduced sporing up to 18 months after application.

The incidence of leaf-scar infection was diminished by treatments reducing sporulation, such as spraying with phenyl mercury chloride or the removal of diseased material from the tree. Bordeaux mixture used as a protectant winter spray was ineffective in reducing leaf-scar infection.

INTRODUCTION

Studies on the development of apple canker, caused by *Nectria galligena* Bres. (Wiltshire, 1921; Marsh, 1939; Crowdy, 1952), have indicated that there are two main paths of entry into the host; leaf scars and pruning wounds. There appear to be two critical periods for the initiation of leaf-scar infections: at the time of leaf-fall and at bud-burst. Pruning cuts show a natural resistance to infection after 2 months. Control might be effected by the use of chemicals which either suppress sporulation or destroy established infections; in the former case protection must be given at the critical periods in autumn and spring. Further the spray must be non-damaging to trees with leaves still attached.

The use of sprays to eradicate established leaf-scar cankers has not been studied but Zeller (1926) noted that a copper spray applied one week after inoculation was effective in reducing leaf-scar infection. A week was the longest period which he used and this is unsatisfactory, as susceptibility to infection continues throughout leaf-fall, i.e. for 6-8 weeks.

Few eradican fungicides have been successfully employed against other pathogenic fungi. They include monocalcium and sodium arsenites (Wilson, 1942), sodium dinitro-*ortho*-cresolate (Keitt, 1939) and sodium pentachlorophenate (Wilson, 1950). Byrde (1950) found each of these materials, and also phenyl mercury chloride, approximately equal in inhibiting the sporulation of *Sclerotinia fructigena* Aderh. & Ruhl. on detached, mummified plums. A preliminary account of the

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trials against apple canker has already been published (Byrde, Crowdy & Roach, 1952).

MATERIALS AND METHODS

The eradican fungicides used in the following trials were phenyl mercury chloride and sodium pentachlorophenate. Copper fungicides have been shown to be highly toxic to *Nectria galligena* (Marsh, 1939) and some attempts were made to distinguish between eradican and protectant effects by including conventional copper sprays. The spray materials were formulated as follows:

Phenyl mercury chloride, by dilution to 100 gal. of a stock suspension formed by milling 3 lb. of the active chemical with 3 gal. of 10% sulphite lye. Oil emulsion or wetting agent was added to the dilute wash if required. In one experiment a proprietary wetting agent at a final concentration of 1% was used instead of sulphite lye.

Sodium pentachlorophenate, as a 1 or 0.5% aqueous solution.

Bordeaux mixture, prepared in the usual manner.

The means of evaluating the treatment varied with the object of the trials. Where the fruiting of the pathogen was assessed a random selection of cankers on the treated trees was examined and classified arbitrarily into five groups, numbered from 0 for those cankers which showed no sign of activity to 4 for cankers which were fruiting freely. From these records a mean fruiting index was calculated by dividing the sum of the individual indices in each treatment by the number of cankers recorded. The maximum possible mean fruiting index is, therefore, 4.

The effects of treatments on the incidence of leaf-scar cankers were assessed by counting in each treated tree the cankers occurring on approximately 100 random shoots of the particular age selected. Usually these shoots were those formed during the season immediately prior to spraying and the probable time at which infection took place could be related fairly accurately to the time of treatment. The results of these counts are presented as the number of cankers per 100 shoots.

In some of the eradican trials, sprays were applied to trees on which a certain number of leaf scars had been inoculated artificially. In such inoculations (Crowdy, 1952) a drop of an aqueous suspension of conidia was placed on a freshly exposed leaf scar, the spores being sucked into the tracheids. Ten leaf scars were inoculated in this way on ten to fifteen shoots on each of the treated trees.

EXPERIMENTAL RESULTS

Effect on young shoot infections

In testing the effect of sprays on recently established infections a number of shoots on trees in the field were inoculated at leaf-fall. The trees were sprayed during the winter and the infection of the inoculated shoots was assessed the following summer. The results of two trials on the varieties Lord Lambourne and Laxton's Superb are summarized in Tables 1 and 2. The Lord Lambourne trees were inoculated in November 1948, sprayed in March 1949 and assessed in May 1949. At assessment

an equal number of inoculated and uninoculated shoots were examined. Four trees were included in each treatment.

Table 2 illustrates a similar trial on Laxton's Superb carried out in the season 1949-50. The times for inoculation, spraying, and assessment were approximately the same as in the previous trial, but three trees were used for each treatment. This method of inoculation is liable to lead to very variable results so two sets of unsprayed controls were included to give some measure of this variability.

TABLE 1. *The direct effect of winter spraying with 0.3% phenyl mercury chloride on leaf-scar infection of Lord Lambourne*

Treatment	Mean cankers per shoot	
	Inoculated	Uninoculated
Phenyl mercury chloride	7.4	0.1
Phenyl mercury chloride and wetter	6.2	0.1
Control, unsprayed	8.7	0.1

TABLE 2. *The direct effect of winter spraying on leaf-scar infection of Laxton's Superb*

Treatment	Mean cankers per 100 shoots
Phenyl mercury chloride, 0.3%	2.2
Sodium pentachlorophenate, 1.0%	15.0
Bordeaux mixture, 10-15-100	0.9
Control, unsprayed A	1.5
Control, unsprayed B	2.7

Tables 1 and 2 show that eradicant winter spraying has little effect in reducing well-established leaf-scar infections.

Prevention of sporulation

Preliminary trials showed that adequate treatment with 0.3% phenyl mercury chloride would completely prevent the formation of perithecia for the whole winter following spraying. This material has been applied to Worcester Pearmain immediately before leaf-fall without causing damage to the mature leaves and shoots, though young autumn-formed shoots may be killed. Phenyl mercury chloride has been used safely when formulated either as an oil emulsion or with sulphite lye or a proprietary wetting agent (Estol H). In the trials recorded below it is probable that the sporulation recorded soon after spraying is a measure of the efficiency of the application rather than that of the formulation. The effect of spraying on the suppression of canker fructifications is illustrated in tables 3 and 4. Table 3 refers to a trial made in a severely cankered orchard of Worcester Pearmain in Gloucestershire. Here there were 3 treatments, each of 16 trees, namely:

Autumn spray, pre-leaf-fall,
Spring spray,
Spray in both autumn and spring.

The trees were sprayed during the autumn and spring of 1950-51 using 0.3 % phenyl mercury chloride in 1 % petroleum oil emulsion. During the winter of 1950-51 a number of cankers, which had been collected at random from unsprayed trees and those sprayed pre-leaf-fall, were examined. Perithecia were readily found on the cankers from the unsprayed trees but none were seen on those which were collected from sprayed trees. The subsequent history of these cankers is outlined in Table 3.

TABLE 3. *The effect of spraying with 0.3 % phenyl mercury chloride on the fruiting of cankers in a Gloucestershire orchard*

Treatment	Date sprayed	Fruiting indices	
		Sporodochia Sept. 1951	Perithecia Feb. 1952
Autumn spray	25. x. 50	0.25	0.19
Spring spray	28. iii. 51	0.58	0.37
Autumn and spring spray	25. x. 50 and 28. iii. 51	0.11	0.21
Control, unsprayed		1.4	0.74

TABLE 4. *The effect of winter spraying on the fruiting of cankers in a Somerset orchard. Sprayed mid-November, 1951*

Treatment	Perithecial index January 1952
Phenyl mercury chloride, 0.3 %	0.08
Sodium pentachlorophenate, 0.5 %	0.50
Control A, unsprayed	1.37
Control B, unsprayed	1.55

Table 4 gives data from a second trial, made on 16-year-old trees of Worcester Pearmain in an orchard at Long Ashton in Somerset. Each treatment included eight trees which were sprayed respectively with 0.3 % phenyl mercury chloride and 0.5 % sodium pentachlorophenate in mid-November, 1951, shortly after leaf-fall was complete.

These data show that eradicator spraying in late autumn or early winter can greatly reduce the fruiting of *N. galligena*. No advantage was gained by delaying the spray application to late in the season. Spraying with phenyl mercury chloride was particularly effective and caused no damage on Worcester Pearmain. The prolonged effect of this spray is also remarkable. It is unlikely that a fungicidal residue would remain for over 12 months on the treated cankers; it seems, therefore, that the suppression of sporulation is due to the killing of the pathogen in the surface layers of the infected tissues coupled with a slow rate of re-invasion. Preliminary observations show that sodium pentachlorophenate will also produce some reduction in fruiting but is far less effective than the phenyl mercury chloride at a comparable concentration.

The effect of suppressing sporulation on the incidence of leaf-scar cankers

During the winter and spring of 1949-50 the following treatments were applied to five groups of Worcester Pearmain trees in the Gloucestershire orchard mentioned above:

- (i) Bordeaux mixture, 16-24-100, applied in late November
- (ii) Bordeaux mixture, 16-24-100, applied in mid-January
- (iii) Bordeaux mixture, 16-24-100, applied in early March
- (iv) Bordeaux mixture, 6-9-100, applied in mid-April
- (v) Bordeaux mixture treatments (i) plus (iv)

In each of these treatment groups six trees were severely pruned and the cankers were cut out. During the following summer the intensity of leaf-scar canker infection was assessed on the treated trees and on two groups of controls. The results are presented in Table 5.

TABLE 5. *The effect of winter spraying and pruning on the incidence of leaf-scar canker on Worcester Pearmain*

Treatment	Mean leaf-scar cankers per 100 shoots	
	Unpruned	Pruned
Control A, unsprayed	60	13
Control B, unsprayed	47	24
Bordeaux mixture, November	71	10
Bordeaux mixture, January	50	23
Bordeaux mixture, March	72	15
Bordeaux mixture, April	61	23
Bordeaux mixture, November and April	38	15

While the winter protectant sprays with a copper fungicide had no appreciable effect on the incidence of leaf-scar canker, such infection was strikingly reduced in the trees where excision of cankers had largely removed the source of spore inoculum. During the following winter (1950-51), groups of sixteen trees in the same orchard were used for a further spraying trial, in which 0.3% phenyl mercury chloride was applied

- (i) before leaf-fall,
- (ii) in the spring,
- (iii) on both occasions.

The consequent reduction in sporulation of the pathogen has been considered above (Table 3). The effect of this reduction on the formation of leaf-scar cankers was assessed in August 1951 and is summarized in Table 6.

During 1951 there were few leaf-scar infections, but the fact that the two series of controls assessed in different parts of the plot show approximately the same degree of infection suggests that the size of sample was adequate. The probabilities that the effects in the sprayed trees are the same as in the controls are shown in the

last column of the table: only the pre-leaf-fall spray gives a probability less than the conventional 5% but in the circumstances the levels of 6% and 7% recorded for the other spray treatments may indicate a genuine effect, in contrast to the results from the protectant sprays used the previous year (see Table 5). Cutting out in 1949 also produced a reduction in cankers in 1951 comparable with that recorded for the spray treatments, but possibly not as striking as that recorded in 1950 (see Table 5). These reductions were produced on single trees in a heavily infected orchard and demonstrate the extent to which the level of infection within a tree controls the incidence of the disease.

TABLE 6. *The effect of suppression of sporulation with a phenyl mercury chloride spray on the formation of leaf-scar cankers*

Treatment	No. leaf-scar cankers per 100 shoots Aug. 1951	Significance level <i>P</i> (%)
Autumn spray	2.0	1
Spring spray	3.3	6
Autumn and spring spray	3.4	7
Pruned and cleaned, 1949	3.6	10
Control <i>A</i> , unsprayed	5.7	—
Control <i>B</i> , unsprayed	5.9	—

DISCUSSION

The results reported above show that winter spraying has no direct eradicator effect on established leaf-scar infections, and that the disease cannot be checked by winter applications of a protectant spray. The data indicate, however, that it is possible in the field to reduce infection materially by reducing the amount of spore inoculum either by cutting out diseased tissue or by the application of eradicator sprays. Two materials, sodium pentachlorophenate and phenyl mercury chloride, have been used; both reduce the sporulation of cankers for some time after application, phenyl mercury chloride being the more effective. The data show further that phenyl mercury chloride owes its effectiveness in reducing leaf-scar canker to the suppression of sporulation rather than to any protectant action, since the results of spraying with this compound are comparable to the results obtained by cutting out diseased material and are far superior to the effects recorded with protectant copper sprays.

Previous observations on the ecology of the disease have shown that the critical times for leaf-scar infections are the autumn and the spring and for pruning wound infection, the winter. In the trials described above the critical period for leaf-scar infections was in the spring but it would be unwise to design a spray programme which took no account of the leaf-fall infection period. However it can be seen from Table 6 that a phenyl mercury chloride spray applied early enough to be effective on leaf-fall infections reduces spring infections as much as one applied later in the season. This provides additional evidence that the early spray inhibits

the production of spores throughout the winter and would, therefore, reduce the infection of pruning wounds as well as of leaf scars.

The economic value of including an eradicant spray against canker in normal orchard routine depends largely on the age of the trees. A routine pre-leaf-fall spray would probably do much to protect the trees in nurseries and young plantings from a serious outbreak of canker and so would allow the main framework of the tree to be built up without hindrance. Eradicant spraying alone, however, is unlikely to be satisfactory in rejuvenating an old and cankered orchard. Such treatment might prevent new infections but the existing cankers would retain living mycelium which might eventually ring and kill the infected branches. Therefore, spraying should be supplemented by excision of cankers.

These trials indicate the potential value of an eradicant spray in the control of apple canker but as the experiments have dealt almost entirely with one material tested on a single variety of apple, wider tests of phytotoxicity are required before the treatment can be generally recommended.

The authors acknowledge with gratitude the assistance and co-operation of Mr N. Rimmington, the owner of the trial orchard in Gloucestershire, the interest shown by Mr R. W. Marsh of Long Ashton and Mr L. Ogilvie of the N.A.A.S. and members of his staff, during the course of the investigation and the assistance of Miss M. Marlow and Miss P. Tozer in recording the results.

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SCAB, CANKER, AND BRANCH BLISTER OF APPLE AT EAST MALLING IN RELATION TO NUTRITIONAL TREATMENT OF THE HOST

BY M. H. MOORE AND MARGERY BENNETT

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On the sandy loam at East Malling, moderate annual dressings of inorganic sources of nitrogen and potash exerted no consistent influence on the incidence of apple scab, even when the dressings were continued over an eleven-year period on established trees of four different varieties on M.IX rootstock. Likewise, no influence of such dressings continued over five seasons was shown on the incidence of apple canker on the rootstock M.VIII, which was grown under conditions favourable for severe infection.

There was evidence from early observations that grassing-down to excess can reduce the incidence of scab, but only at the expense of nitrogen starvation in the trees. Short-term mixed leys had no such effect.

The incidence of branch blister, a functional disease associated with adverse soil conditions and retarded vigour, was greatly reduced in the variety Cox's Orange Pippin by potash manuring, and was further influenced by the rootstock.

I. APPLE SCAB (*VENTURIA INAEQUALIS*)

Early observations, 1929-42

In the very dry season of 1929, cultivated Bramley's Seedling and Worcester Pearmain trees at East Malling showed much more scab-infection when manured only with green crops grown *in situ* and ploughed in than similar trees receiving balanced manurial treatment (Moore, 1930). There was no evidence to show to what the difference could be ascribed. The incidence of apple mildew (*Podosphaera leucotricha*), though, like scab, responding to the influence of the differing rootstocks on which the trees were worked, was unaffected by the differential manuring.

Further observations (Moore, 1936), following changes in the experimental design (Hoblyn & Bane, 1934), indicated the complexity of manurial influence when involved with interacting factors such as scion variety, rootstock, and weather conditions. The chief response to scab was shown after some of the trees had been grassed-down for two years without any ameliorating nitrogenous manuring. This treatment proved drastic enough to result in symptoms of severe nitrogen deficiency, and the incidence of scab was reduced, especially on Worcester Pearmain, by comparison with that on similar cultivated trees or on those that had been under grass for only one year.

From 1934-42, various pomological experiments concerning the effects of manuring, and manuring added to cover-crop treatments, were examined for any influence shown on scab. Inorganic manurial treatments on cultivated land (Hoblyn, 1941)

and short-term mixed leys (Rogers & Raptopoulos, 1945) showed no influence, and, from a study of long-term leys (Rogers, Raptopoulos & Greenham, 1948) only one significant result emerged. In 1941, Worcester Pearmain fruits from trees in grass infrequently cut showed less scab than those from trees in grass frequently cut (Table 1); long grass would tend more than short to rob the trees of nitrogen (Rogers *et al.* 1948). A similar comparison in 1942 showed no significant difference.

TABLE 1. *Incidence of scab* on Worcester Pearmain in relation to length of grass, 1941*

Proportion of clovers in grass mixture:	Sward cut infrequently	Sward cut frequently
<i>A</i> (low)	16.5	22.4
<i>B</i> (high)	15.0	22.8
Mean	15.7	22.6

* As scab 'equivalent' (Moore, 1930).

In these experiments the presence of scab interfered with the pomological issues, and the disease was controlled as much as possible by spraying, so that infection was usually low and differences were small. In 1938, therefore, a long-term experiment was designed expressly to investigate any relationship between the incidence of scab and moderate inorganic manurial dressings (Nil *v.* N *v.* NK), and to avoid complication, trees on only one rootstock (M. IX) were included.

A. *Experiments on established trees, 1938-48*

Materials and method

Two-year-old trees of Worcester Pearmain, Edward VII, Allington Pippin, and Newton Wonder, were planted 12 ft. square in 1929 in seven 4 × 4 Latin squares on each of two neighbouring plots. One of these (Plot 15) had hitherto grown plums and black currants, and the soil had therefore been well manured. The other (Plot 18), with apparently similar soil, had for many years accommodated a rootstock nursery, which makes heavy demands on the soil. In spring, 1937, the Edward VII trees were framework-grafted to the more scab-susceptible Cox's Orange Pippin. The manurial experiments were started on Plot 18 in 1938 and on Plot 15 in 1940; the same manurial comparison was replicated five times on each plot. Two of the five replicates on Plot 18 suffered war damage in 1940, and were of no further service in these experiments.

The source of nitrogen (N) was sulphate of ammonia applied annually at 3 cwt. per acre as two half-dressings in February and May. Sulphate of potash (K) was applied annually at 3 cwt. per acre in winter. Only every alternate transverse column of four trees received manure, in a strip extending to the trunks of the intervening (buffer) trees on either side. The plots were cultivated till midsummer, after which natural weeds were allowed to develop as a cover-crop, which was disked under in spring.

Spraying was designed to provide partial scab control, as unchecked infection might have ruined the trees for experiments after several years. This device largely failed, however, because of unpredictable seasonal conditions; the disease in fact varied greatly in severity from one year to another in spite of the efforts made to keep it reasonably constant. Methods tried included the use of a normal schedule of sprays each at reduced strength, and of an incomplete schedule of sprays at reduced and at full strength. It is probably best to withhold the later scab sprays, but even so, much dry weather in summer may vitiate this provision for moderate infection.

Scab-infection on leaves was assessed by grading the lesions on the upper surfaces of all leaves on sample shoots chosen at random, and on the fruits (Moore, 1930; Moore & Pearce, 1946). The results were expressed as severity of scab (percentage scab $\times$ mean grade).

Results

Mean values were examined statistically, the data concerning fruits and leaves being taken together. There was no significant relationship between manurial treatment and the incidence of scab, or the cropping of the trees, or the percentage increase in their girth (measured 9 in. above the graft union) when the period was considered as a whole. In the year-by-year analyses, the few significant differences

TABLE 2. *Vigour (as girth), and incidence of scab on leaves and fruits, 1938-48**

	Worcester		Cox's		Allington		Newton	
	Scab	Mean girth (cm.) 1948	Scab	Mean girth (cm.) 1948	Scab	Mean girth (cm.) 1948	Scab	Mean girth (cm.) 1948
Plot 15	49.7	26.9	80.1	28.6	71.5	34.6	94.9	34.2
Plot 18	48.1	19.9	67.0	21.4	62.3	28.9	79.3	25.3
Mean scab for variety	48.9		73.6		66.9		87.1	

* Including all manurial treatments.

as to cropping and the incidence of scab showed no consistency for any one manurial treatment, however, and may therefore have been due to chance or to a temporary seasonal influence on the effects of manuring. Only four significant differences for scab occurred in a total over the years of forty-four comparisons for Plot 15, and one in forty-eight for Plot 18.

A comparison of actual girth measurements (Table 2) showed that the trees of all varieties grew more vigorously and also, except for Worcester, showed more scab on Plot 15 than on Plot 18. In these experiments, therefore, an initial difference in soil conditions was a factor of more weight than the manurial treatments.

Scab-infection on Cox's and Allington was significantly greater than that on Worcester, and less than that on Newton on both plots, in spite of the complication of war damage on Plot 18. Evidently the variety, like the soil, was a more telling factor than some ten seasons' cumulative differential manuring.

B. *Experiments on unworked rootstocks, 1944-6*

When it became clear that the established trees would respond but slowly, if at all, to the moderate differential treatments applied, tests were begun on young apple rootstocks to see whether the response would be enhanced by virtue of their more compact root-system, and to test the effect of differing levels of nitrogenous dressing. Experience had shown M.III to be scab-susceptible, and the crop of young shoots produced every year from cutting the previous year's crop to the ground in winter is specially suitable for infection (Moore, 1952), and convenient for sampling, detailed observation, and recording.

Materials and method

A 240 ft. row of M.III stocks, planted about 1 ft. apart in December, 1941, was in 1944 divided into 10 ft. plots, and six replicates of four randomized manurial treatments were designed. Sulphate of ammonia as an inorganic source of nitrogen was applied to the treated plots in March each year (from 1944) within a strip 1 ft. wide each side of the row. Three levels of dressing, at the rate of 2 cwt. (N), 4 cwt. (2N), and 6 cwt. (3N) per acre, with an untreated control (Nil), comprised the four treatments.

Results

Natural scab-infection was recorded by grading the lesions on the foliage of randomly-chosen shoots every summer (omitting 1 ft. at each end of every plot for any 'overlap' effects), and the results are expressed in Table 3 as severity of scab. Infection in 1944 was slight and irregular, and the data are not presented.

TABLE 3. *Incidence of scab on leaves of M.III rootstock in relation to manurial treatments, 1945-6*

Treatment	Severity of scab	
	1945	1946
Nil	9.7	90.1
N	20.0	127.3
2N	15.2	104.3
3N	30.5	116.6

Differences were not significant owing to variation in response among the replicates. In both seasons, and especially in 1946 when scab was very severe, the plants in eight contiguous plots in the middle of the row showed much less infection than their fellows, whatever the manurial treatment. The aberrant plots may have been affected by subsoil thrown out from a nearby well years before, for the mechanical nature of the surface soil around them was clearly different from that in the rest of the row. Nevertheless, neither auger borings in the lower layers nor chemical analysis of leaf samples shed any light on this matter, and further investigation of the manurial issue was therefore abandoned on this rootstock

material. The leaf analyses along the row showed a general deficiency of magnesium (a possible limiting factor) that was not related to the level of nitrogen dressing, but the manganese content was increased by 3N.

II. APPLE CANKER (*NECTRIA GALLIGENA*)

There is much speculation but little evidence in this country that manurial treatment influences the incidence of canker; the rootstock, however, has a marked effect (Moore, 1934; Rogers, 1946). In comparing the performance of trees on five different soil series in Kent, Rogers (1946) showed that 'the incidence of canker was clearly related to soil type'. Trees on clay or compact loams were severely affected by canker and scab, and similar ones on well-drained brick-earths were not. Earlier, Bane & Jones (1934), in a survey of the soils and fruit in the fruit-growing areas of the Lower Greensand in Kent, had noted the prevalence of canker in trees on certain soil-types. These were usually, though not invariably, associated with some degree of impeded drainage, one notable exception being the Chart Series of the Ragstone formation, where, however, the soil was often very shallow because of erosion. The writers remark that in this case 'it is not clear whether this (severe canker) is the result of soil conditions or of an excess of nitrogenous manures applied in the belief that these shallow and dry soils need much feeding'. In another instance, concerning the rather poorly drained, low-lying Dean Series, 'a very severe attack of canker was noted in a plantation of apples making rather rank and soft growth, but whether the cause lies in the water supply of the soil or in manurial treatment, or in both, is not clear'. A possible indirect effect of soil conditions should not be overlooked, for it is very likely that canker spores would flourish in the moist air overlying poorly drained soil, especially if this were in a sheltered situation.

Stahel (1946) found that excessive nitrogen and severe pruning induced soft growth conducive to canker in apple, and McKay (1947) reported an instance from Eire in which susceptibility in a Lord Derby apple tree had apparently been induced by excess nitrogen from a heap of farmyard manure left a few yards from the tree.

To provide evidence for any relationship there may be between inorganic manurial treatment and the incidence of canker, an experiment was begun in 1943. The apple rootstock M. VIII, known from observation to be susceptible to the disease, was chosen as the host; as with scab, a susceptible rootstock yields readily to infection when compactly growing in the humid conditions that are found or can be promoted and maintained near the soil, and when liberally provided with inoculum.

Experiments on unworked rootstocks, 1942-6

Materials and method

A row of M. VIII rootstocks was planted in the shelter of a building in spring, 1942, and a manurial experiment* was started including four replications of four

* In co-operation with Dr W. S. Rogers.

randomized treatments (Nil, N, NK, K), each being applied to a 9 ft. length of row. Sulphate of ammonia (N) at 6 cwt. per acre was sown in two equal dressings in February and May, and sulphate of potash (K) at 3 cwt. per acre in one winter dressing every year. The manured strip extended 3 ft. each side of the row.

Early in December 1944, when the stocks were well established and had been differentially manured for the previous three years, cankers bearing abundant perithecia containing viable ascospores were tied among the young branches as a source of infection. The stocks became freely infected, and at the end of 1945 all new cankers were cut out and recorded. Suitable specimens from among them were re-distributed uniformly among the branches in January, 1946. By the end of 1946 all further new infections were recorded, but so many of the shoots had by then been killed by the disease that the experiment had to be abandoned.

This rootstock produced numerous aerial 'burrknobs' (Hatton, Wormald & Witt, 1926), which are callus-like outgrowths bearing adventitious root-initials. Their frequency was recorded in relation to manurial treatment, and to canker infection in 1945, as some of them were seen to be infected.

In March, 1947, all shoots were cut to the ground and their weight was recorded. This value, together with that for all prunings (as cankered shoots already removed) gave an index of total vigour. The total number of cankers per treatment was obtained for the two years at the end of the experiment.

Results

There were many more cankers where potash was applied than where it was not (Table 4), but, as potash is a limiting factor on East Malling soil, there was also more growth. When the total number of cankers was corrected for growth weight, there was no significant influence of the manurial treatments on infection.

TABLE 4. *Incidence of canker on M. VIII rootstock in relation to manurial treatment, 1945-6*

	Nil	N	NK	K
Gross top weight (lb.), 1942-6.	56.0	58.9	68.9	76.2
Ratio: No. of burrknobs/wt. of prunings, 1945-6.	51.1	52.9	33.9	44.8
% cankered burrknobs, 1945.	13.4	14.1	12.0	14.8
Total no. cankers, 1945-6.	150	144	272	222
Ratio: No. cankers/lb. gross top weight, 1942-6.	2.7	2.4	3.9	2.9

The greater production of burrknobs per unit weight of prunings in the absence of potash was not a significant difference; manurial treatment also had no significant influence on the cankering of the burrknobs in 1945, and this value was therefore not recorded in 1946.

III. APPLE BRANCH BLISTER (FUNCTIONAL)

Small, bullate, purplish blisters, sometimes massed together, may often be found on the younger bark of apple trees that are growing poorly; the bark also may be of unusual colour (yellowish or dull red) denoting an unhealthy condition. In early work on this disease, which was described and illustrated (Moore, 1931), attention was drawn to the association with these symptoms ('apple branch-blister,' van der Byl, 1914), and with fruit-russetting and cracking, of a fungus tentatively referred to as *Coniothecium chomatosporum* Corda, and the reports of other workers as to its pathogenicity were quoted. Later, numerous inoculation experiments with pure cultures of the associated fungus all yielded negative results.

Incidence on Cox's trees in relation to manuring and rootstock, 1935

In 1935, an opportunity occurred to obtain data on the presence of branch blister on young Cox's trees undergoing differing inorganic manurial treatments applied annually since 1931 to soil that had been deliberately impoverished and, though incidentally, probably impaired in texture by previous treatment (Hoblyn, 1941). The blister had developed during a series of dry summers, and a general

TABLE 5. *Incidence of branch blister in Cox's in relation to manurial treatment and rootstock, 1935*

Rootstock	Nil	N	NP	P	K	NK	PK	NPK	Mean for stock, K absent
I	1.4	1.4	1.6	1.4	0.0	0.0	0.0	0.0	1.5
V	0.6	0.3	0.4	0.5	0.0	0.0	0.0	0.4*	0.5
IX	2.1	2.1	2.4	2.0	0.3	0.4	0.6	0.2	2.2
XII	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	K absent:		1.0	K present:		0.1			

* Present on one tree only.

survey of the plot indicated differences in relation to manurial treatment. Records were therefore made by allotting a category figure 0-3 to each tree according to the severity of the blistering. The eight manurial treatments (Table 5) were arranged in randomized plots in each of eight blocks. These sixty-four severally manured plots each contained one Cox's tree on each of the rootstocks M.I, M.V, and M.XII, and two on M.IX, i.e. 320 trees in all were assessed for blister. The data are expressed as mean category values per tree.

An effect of potash in controlling blister is obvious, as is the influence of rootstock. Trees on M.IX, severely blistered without potash, were, with the exception noted, the only ones even slightly affected where potash was applied. Lack of blister was associated with tree growth, for potash manuring promoted vigour in this experiment (Hoblyn, 1941); moreover, none of the trees on the vigorous M.XII showed any blister.

DISCUSSION AND CONCLUSIONS

I. *Apple scab*

Johnstone (1931) showed that when Bramley's Seedling and Worcester Pearmain trees on M. IX in pots, subjected to differing nutritional treatments, were inoculated, 'a lower proportion of infections occurred in trees of both varieties which were deficient in nitrogen or received rainwater only'. Moreover, trees from which potash dressings were withheld showed increased susceptibility, a fact that may bear on the results obtained at East Malling in 1929 (see p. 588). Holz (1939) found that nitrogenous manuring increased the susceptibility of apples to scab, and that potash dressings decreased it. Palmer (1947) records an experiment in which soil applications of an urea preparation, when compared with applications to the leaves in spray form, increased the incidence of scab on the McIntosh apple. Muskett, Horne & Colhoun (1938) showed that nitrogenous manuring in excess of normal practice increased the nitrogen content of Bramley's Seedling apple fruits in Northern Ireland, and that such fruits were the more infected by scab.

In the eleven-year period at present under review, however, *moderate* annual dressings of sulphate of ammonia and sulphate of potash had no consistent effect on the incidence of scab. The host variety and a wide contrast in recent soil history were the only material influences observed. An experiment on apple rootstocks to test the effect of differing levels of nitrogenous manuring was inconclusive. The soil is a sandy loam river drift overlying the Hythe Beds in the Lower Greensand Series, and tends to lack humus and potash. Though prolonged differential manuring would be expected to influence the nutritional status of the soil and the trees, any such influence was not reflected either in cropping or the incidence of scab. This may not hold good for all soils or stock/scion combinations; Rogers (1946) showed that trees on M. IX were relatively unresponsive to environmental changes, and Muskett *et al.* have shown that excessive manurial treatments affect scab susceptibility under conditions in Northern Ireland. Sometimes an excess, such, for example, as heavy dressings of sulphate of ammonia or prolonged sooting by pigs, will promote vigorous, dense growth that dries slowly after rain or dew and is difficult to penetrate with a spray. These conditions are favourable for scab, and the trees are thus the more liable to become infected as well as possibly the more susceptible in the sense suggested by Johnstone and by Muskett *et al.* A converse effect of an excessive treatment has already been cited, when grassing-down without corrective nitrogenous treatment led to symptoms of severe nitrogen deficiency and low incidence of scab.

II. *Apple canker*

The available data for apple canker are much less extensive, but they point towards a similar conclusion. It was not expected that the rootstocks receiving potash would show much more canker than those without it, because potash is regarded as a corrective for soft, rapid growth, usually regarded as especially

susceptible to canker. However, lack of potash tends to be a limiting factor to growth on East Malling soil (Hoblyn, 1941), so the potash dressings increased the vigour of the stocks receiving it, thereby increasing the size of the target presented to the spores. No account was taken of the sizes of the cankers—a problem in interpretation already discussed for established trees (Moore, 1934), but less intrusive on young rootstocks where there is no such great divergence in age and size of shoots as there is between twigs and branches of established trees.

After due correction had been made for the vigour of growth expressed as gross weight of the stocks above ground, there was no evidence of any effect of these moderate manurial treatments on the incidence of infection, for a difference in favour of NK was not significant. Neither was the presence of the burrknots of significant importance for cankers.

Further point is given to the distinction between liability and susceptibility to infection by these canker experiments. Obviously, the M.VIII rootstocks were susceptible, but they remained uninfected for three years until inoculum was provided by tying-in fertile cankers; then they quickly succumbed. Conversely, trees in a neglected plantation, though susceptible, may be especially liable to infection because of past neglect in removing old cankers, which are a prolific source of mounting infection. The management factor must be considered before observed numerical differences in canker from one plantation to another could confidently be ascribed to differing nutrition.

III. *Apple branch blister disease*

It seems clear that the fungus *C. chomatosporum* Corda is merely associated with, and is not the cause of, the disease. The conclusions of Australian and New Zealand workers (see Moore, 1931) that nutritional deficiency is primarily responsible, and that measures to promote normal vigour are of first importance in avoiding the disease, are supported by the present evidence. Although trees on the very vigorous M.XII showed no blister, not all potentially vigorous trees are proof against it, for it was seen many times in Essex and elsewhere on young Cox's on M.II when attempts were being made to establish the trees under dry and sandy or gravelly soil conditions. This suggests that one limiting factor may be soil moisture, shortage of which may accentuate, or be accentuated by, a nutritional deficiency (e.g. at East Malling, potash) of the locality concerned. Support for this hypothesis was forthcoming from soil-moisture experiments made later on young trees in pots and not yet reported; moreover, it has long been known for cereals that potash dressings can ameliorate an adverse influence of dry seasons (Hall, 1921). There may be no conflict in postulating that potash deficiency in relation to branch blister at East Malling and boron deficiency in relation to 'apple measles' in Queensland (Thomas, 1944) are alike contributory to a similar physiological disorder that probably stems from dry as well as impoverished soil. Vigorous trees seem better able than more weakly ones to withstand such adverse conditions. The rootstock

plays a part, less perhaps because of the range than of the quality of its root system, for the root-range of a tree even on the dwarfing M. IX may be extensive and deep, though relatively small in mass (Rogers & Vyvyan, 1934).

Branch blister is often associated with russetting and cracking in the fruit of affected trees (Moore, 1931). These symptoms also are probably due to the same conditions, directly because of the toughened and undersized fruits usually yielded in drought, and indirectly because of the sparse foliage, which does not shade the fruits from the sun (Moore, Steer & Shaw, 1939). The shading experiments reported by Louw (1948), working with Ohenimuri apples in S. Africa, support these views. He suggested that the cracking may be due to excessive loss of moisture from the outer tissues of the fruits in conditions of low relative humidity. Symptoms of russetting and cracking in relation to weather conditions were investigated by Tetley (1930) and by Verner (1938). These workers concluded that cracking is preceded by localized restriction in the growth of the outer tissues of the fruit, which later give way under growth pressure from the underlying tissues. Blister and fruit-russetting are, however, not invariably associated; russetting may sometimes occur from other causes such as spray damage or early frost (Moore, 1931).

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ACUTE DIE-BACK OF CLOVE TREES IN THE ZANZIBAR PROTECTORATE

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(With Plates 21 and 22)

The most widespread die-back of clove trees (*Eugenia aromatica*) in the Zanzibar Protectorate is caused by *Cryptosporella eugeniae* sp.nov. The most noticeable symptom is the death of a branch or a portion of a branch, or, in young saplings, of the entire tree; in mature trees infection eventually leads to the semi-moribund trees now common in almost every clove plantation. The fungus invariably enters through a wound, most often one resulting from harvest damage. Pycnidia, and later perithecia, develop near the point of entry. Internally the infected wood is clearly distinguishable from the healthy by a dark red-brown stain.

Experimental infections that reproduce typical symptoms can rarely be caused in plants aged 18 to 36 months, and not at all in young seedlings. Experimental infection of older plants becomes more certain with increasing age, and with saplings of 7-10 years old failure is rare.

The prevalence of *Cryptosporella* die-back is almost entirely attributable to the destructive methods of harvesting now practised, combined with the presence of much infected material present in the plantations. Suggested control measures are based on the removal of old sources of infection and prevention of future damage, combined with remedial treatment where possible.

A group of diseases, known collectively as 'die-back' of clove trees, is widespread in the Zanzibar Protectorate; the form described here, distinguished as '*Cryptosporella* die-back', probably causes even greater loss of crop than does the sudden-death disease (Tidbury, 1949). Although 'die-back' has been known for perhaps fifty years, little is known about its history; no experimental work has been done and the few accounts which exist often do not describe symptoms in sufficient detail to give any certainty about which kind of die-back the writer meant. Moreover, there is no doubt that some forms of *Cryptosporella* die-back have in the past been confused with sudden-death.

Fitzgerald (1895) and Campbell (1940) both described as 'die-back' a gradual decline of immature trees, characterized by a progressive reduction of foliage over many months. This condition, or a similar one, was thought by Nutman & Sheffield (1949) to be one form of sudden-death; the cause is still unknown and is at present being investigated. It seems invariably to be associated with a root-rot.

Dowson (1914), Troup (1932) and Wigg (1937) described a dying back of the extreme tips of young twigs and attributed this to various causes, such as badly drained soil, cockchafer damage to the roots, and competition from grasses. Present

* Seconded from Rothamsted Experimental Station.

investigations, however, make it seem likely that a similar form of small twig die-back, often found on mature trees and now common in the Protectorate, is of fungal origin. The terminal twigs are invaded through wounds resulting from the harvesting of the inflorescences. At least two mild (unidentified) pathogens have been isolated, but the great majority of such effects seem to be caused by a species of *Valsa*.

The form of die-back described in this paper is easily recognizable and is the most common and important in the Protectorate. It is invariably caused by a species of *Cryptosporella eugeniae* sp. nov. Nutman & Roberts. This fungus will be described in detail elsewhere, but a formal diagnosis is appended to this paper in order to establish the name. Welsford (1922) recognized and correctly described what we know to be *Cryptosporella* die-back, but suggested that the condition might be associated with a fungal root-rot in some trees, or a leaf-spotting fungus in others. Campbell (1940) also distinguished between this and other forms of die-back but suggested that unfavourable water relations might be the cause.

DESCRIPTION OF THE DISEASE

The first obvious sign of attack by *C. eugeniae* is that a part of a tree dies suddenly. Before this happens, however, pycnidia can be found (Plate 21, fig. 4); their necks protrude from the bark around the point of entry of the fungus—usually a wound produced at harvest. Perithecia, difficult to distinguish from pycnidia except microscopically, appear later. Neither are conspicuous, and both have escaped the notice of previous workers. In damp conditions yellow spore tendrils are extruded, and these become distributed over the adjacent bark, giving it a brownish-orange tinge. In thick bark, where the pycnidial necks do not always reach the surface, the only external signs, seen only under suitable moisture conditions, are tendrils of pycnospores, sometimes 1 cm. or more in length, which are extruded through cracks.

The general symptoms are similar on trees of all ages, but the effect of infection varies with the point of entry of the fungus. When, as often with young saplings, invasion occurs in the lower part of the trunk, the whole plant dies suddenly with most of the leaves still adhering and drying to a bright russet colour (Plate 22, fig. 8). A similar effect has occasionally been observed when infection has been through the roots, probably because of damage to exposed surface roots during cultivation. These symptoms are precisely those of the sudden-death disease, and hence attack by *C. eugeniae* on young trees has been mistakenly diagnosed as sudden-death (Nutman & Sheffield, 1949). Campbell (1940), by the evidence of a colour photograph which he attached to his report on sudden-death, also made the same error.

In young to fully mature trees, infection can take place where any damage has occurred; this is usually a branch broken deliberately during picking by the methods now practised in the Protectorate (Plate 21, figs. 1, 3 and 4; Plate 22, fig. 7). The distal part of the branch dies suddenly and the fungus then progresses towards the

main trunk, lateral branches on the limb dying successively (Plate 21, figs. 1a and 1b). This eventually results in the production of the 'stag-headed' trees, with a number of dead limbs, which are to-day a most conspicuous feature of the clove plantations (Plate 22, figs. 5 and 6).

Irrespective of where the fungus enters the tree, sooner or later it reaches the main trunk. The whole tree may die because the trunk is girdled, when the condition is wrongly diagnosed as sudden-death. Or incomplete girdling may give almost skeleton trees, unsightly and of no economic value, which still retain one or two living branches connected to the roots by bands of healthy tissue (Plate 22, fig. 6).

When an affected branch is slashed the infected wood is seen to be sharply demarcated from the light-coloured healthy wood by its dark reddish-brown stain (Plate 21, figs. 1b and 3). Microscopical examination shows that the mycelium is virtually confined to the stained region and hyphae can be found only with difficulty up to a few millimetres outside this. In this zone, the vessels are completely occluded by tyloses. All tissues appear to be attacked equally, with a profuse production of gum, especially in the medullary rays.*

INOCULATION EXPERIMENTS WITH *C. EUGENIAE*

Mature trees

Trunks, branches, and main lateral roots of mature trees were inoculated, using either pure culture mycelium or spore suspensions. Examination nine months later showed that the fungus was growing in all directions from every point of inoculation. It is certain that in these mature trees infection was established, but they have not yet developed clinical die-back. It depends entirely on the direction of spread whether it is months or years before a main limb becomes cut off and clinical die-back produced. For example, one mature tree was inoculated at two points in the outer bark, each about 1 cm. square and 1.5 in. apart; five months later the fungus had spread for a distance of 20 in. longitudinally, but only 2.3 in. tangentially.

Trunks of young trees

The main stems of twelve trees, 8-12 years old, were inoculated about 2 ft. above ground level with mycelium from a pure culture. A small incision was made in the bark, the mycelium inserted, and the whole covered with wet cotton wool and waterproof Cellophane, which was bound in position and left for ten days. (Later experience showed that these precautions were unnecessary, equally good results being obtained by leaving the wounds unprotected.) Eight trees were in average to good condition at the time they were inoculated, but four had exceptionally dense foliage and were unusually vigorous.

The first pycnidia of *C. eugeniae* were found close to the points of inoculation on

* We have found picro-aniline blue with safranin to be an excellent stain for revealing fungal hyphae in clove wood, the standard cotton blue-lactophenol being most unsatisfactory for this purpose.

some trees after less than 4 weeks. After 8 weeks one sapling was felled and examined (Table 1). The zone of fructifications and intensely stained wood extended 8.2 in. up the trunk and discoloured streaks extended 11.2 in. above and below the point of inoculation. The course of development of the fungus in this and the remaining trees is shown in Table 1.

TABLE 1. *Rate of development of C. eugeniae in 8-12-year-old clove trees inoculated January 1951 and last observed November 1951*

Tree no.	Girth (in.)	Longitudinal spread (in.)	Tangential spread (in.)	Remarks
1	5.9	17.0	4.2	—
2	5.2	8.9	3.9	One adjacent branch died suddenly after 9½ months.
3	6.5	19.0	—	Tree died after 9 months in sudden-death manner.
4	4.5	11.5	3.0	One adjacent branch died after 7 months and two more after 9 months.
5	5.8	15.0	2.8	One adjacent branch died suddenly after 9½ months.
6	—	—	—	Whole tree died in the sudden-death manner after 5 months. The stain extended throughout the trunk and down to the roots.
7	8.8	0	0	This was one of the exceptionally good trees. The wound has apparently healed.
8	8.5	0	0	Another exceptional tree. Only very slight development of the fungus.
9	10.8	0	0	As no. 8.
10	9.1	0	0	As no. 8.
11	4.9	11.5	—	Felled for examination after 2 months*
12	7.8	17.1	3.9	One branch dead after 9 months.

* See p. 602.

Plate 21, fig. 2, illustrates the progress of the fungus, as shown by pycnidial development, in tree no. 3.

The only trees in which initial infection was not followed by disease development were those which had the unusually thick foliage. It may be that an exceptionally vigorous tree can, in some conditions, resist invasion.

Branches of young trees

To simulate the type of injury caused during harvesting, separate branches on twenty 8-year-old trees were used. The branches, of an average diameter of about 0.5 in., were twisted until longitudinal cracks appeared in the bark, or they were bent until the convex side of the bend fractured. The injuries were not excessive as eighteen of these branches (nine for each type of treatment) on six trees were left uninoculated as controls, and 5 months after treatment, these remained healthy.

Twelve of the twisted, and fifteen of the fractured branches, were inoculated with a pycnosporium suspension from a pure culture, by running small quantities of inoculum from a pipette over the injured branches. This was readily absorbed by the exposed wood.

After only 8 days it was evident that the fungus was proliferating in and near the broken tissues, and in one branch it was found 6 in. from the fracture. When small flaps of the outer tissue were raised with a sharp knife, the characteristic stain revealed the extent of spread. After 5 weeks pycnidia developed on nearly all the inoculated branches, and after 8 weeks fourteen out of the twenty-seven were dead. In two of these young trees, the fungus had reached the main trunk. After 4 months all the inoculated branches were dead and bearing fructifications of the fungus.

Young plants

An experiment was made with sixteen 3-year-old plants grown in large containers in a field nursery. Half were inoculated in the main stems 3 to 4 in. above soil level, and half in two lateral roots near the collar. There were sixteen control plants.

Two months after inoculation one inoculated plant in the stem suddenly wilted and died. The stem was girdled and tendrils of pycnospores of *C. eugeniae* were produced on the bark for 2 in. above and below the point of inoculation. The fungus was re-isolated from the dead tissues. At this time, the fungus was also progressing in several other plants.

The plants were then moved to a less shaded position at a time when the breaking of the monsoon brought cooler weather; they soon responded by a vigorous flush of new leaves, and the fungus not only ceased to progress but the originally affected areas started to recover. None of the root-inoculated plants became infected and all the controls remained healthy.

Sixteen similar plants from the same batch were again inoculated, eight in the main stem by the bark-flap method, and eight by drilling a ring of holes of approximately 1 mm. diameter around the main stem and flooding these with a suspension of pycnospores. Three months later one plant inoculated by the first method suddenly died and showed *C. eugeniae* fructifications and stain above and below the point of inoculation; a second plant, inoculated by the bark-flap method, died after 4 months, and a third after 5 months. This experiment still continues and several other inoculated plants are almost girdled by the fungus.

A similar experiment was made with twenty potted clove seedlings, 18-20 months old, which had been grown in a nursery under dense shade, and had reached the stage where no new growth could be expected until after re-potting or planting out. The inoculum was mycelium from a pure culture. Ten were inoculated in the main stem under a bark flap which was afterwards covered with moist cotton-wool and bound back into position, and ten by bending a lateral twig downwards until it fractured when inoculum was inserted. The break was covered as before. An equal number of control plants was left.

After 15 days, two of the plants inoculated in the main stem died from the point of inoculation upwards and a month later five were dead; *C. eugeniae* was

re-isolated from these. The other five plants did not become infected, nor did any of the plants inoculated in fractured twigs.

Microscopical study of these plants showed that the fungus had produced a discoloured zone extending inwards from the ruptured tissues for 3–4 mm., and that it was no longer active. Surrounding the discoloured zone was a band of tissue whose cells and cell walls contained a dark gummy substance.

Very young seedlings

The many attempts to infect very young seedlings in the 2-leaf stage have all been unsuccessful.

Roots

Except in exposed lateral roots of mature trees, in which the fungus develops rapidly from the initial point of inoculation, no positive results have been obtained in any experiments in which roots have been inoculated and re-covered with soil. Field observations, however, show that infection through injuries to exposed roots does occur.

From these and similar observations it is clear that *C. eugeniae* can be an active and virulent parasite on the clove tree. In mature trees, however vigorous, no resistance to infection has been observed. In saplings, aged about 8 years, failure to cause infection is very rare, although it may be that some exceptionally vigorous trees are able to resist artificial infection. In younger plants, up to 3 years of age, resistance is common, and only occasionally can a high proportion of infections be secured experimentally. Finally, in young seedlings of a few weeks or months old, we have failed to secure experimental infection, even when they are severely mutilated.

These results agree with field observations. Young seedlings have never been found infected in the field or nursery, and it is rare to observe natural infections until plants are 4–6 years old. With increasing age, infection in the field becomes common.

D. M. Cooper (unpublished) has made preliminary studies on this effect. Her results suggest that aqueous extracts from the bark of young clove plants may depress germination and growth of *C. eugeniae* whereas extracts from mature trees do not.

DISCUSSION

Distribution and economic importance

Die-back occurs in all parts of Zanzibar and Pemba. It is unfortunate for the clove industry that, because of the more spectacular nature of sudden-death disease, die-back (except where this has been mistaken for sudden-death) has caused little concern and is scarcely recognized by the average grower as a serious threat. It not only slowly kills mature trees, but also kills young ones rapidly. Many mature plantations are already affected, and, if not now uneconomic, soon will be, and young plantations designed to replace them are suffering increasing casualties.

Cultural methods in relation to spread

To understand why die-back has reached its present intensity, the cultural methods practised in Zanzibar need to be considered. The clove is a large evergreen tree, coming into bearing at about 9 years of age, but not producing an appreciable crop until about 20 years old (Tidbury, 1949). The crop of unopened flower buds is borne terminally on the twigs and so is peripheral on the tree. It is picked by labourers who climb the tree and pull the branches inwards by ropes and hooks. Under the slave economy of the past, picking was carefully supervised and damage to the trees was visited by severe punishment. At present, most of the plantation owners are absentee landlords. The unharvested crops are often leased to a speculator whose sole interest is to obtain the maximum return, and who has no regard for the trees. The pickers are paid on quantity harvested, and have every incentive to pick as rapidly as possible, regardless of damage to the trees. They generally work with their families and, to increase speed, the men climb the trees and cut and break flowering branches which are thrown to the ground and stripped there by the women and children. Thus, after a harvest, very many open and ragged wounds are left on every tree (Plate 21, fig. 4; Plate 22, fig. 7). These wounds become infected with *C. eugeniae*, often at many places on the same tree, and the effect of these new infections is obvious three months or so after harvest. Younger trees, up to bearing age, suffer no harvest damage, but infection, usually in the main trunk, results from such causes as careless cultivation, abrasions caused by tethering animals to the trees, or from the practice of deeply trenching the ground in young plantations for cassava intercultivation. In every one of the thousands of local infections we have examined, whether in mature or young trees, the wound through which the fungus obtained entry was always obvious.

Two examples of the rapid degeneration of formerly good plantations after harvest damage can be cited. An unusually good Government plantation suffered severe picking damage during the 1927-8 harvest. By 1932 it was recognized as suffering from die-back and, since the cause was unknown, various ameliorative cultural procedures were tried, although without success. By 1939 the plantation was becoming uneconomic and at present, by 1951, few of the original stand remain alive, and these are all moribund (Plate 22, fig. 6). A high proportion of the saplings supplied to fill the vacant sites are suffering from attack by *C. eugeniae*.

The second example, an isolated and well-cared-for plantation which we had been observing for some time, was in exceptionally good condition until the 1950-51 harvest, and there was little die-back. Following a change of ownership and a heavy crop the trees suffered picking damage, although on ordinary standards this was not excessive. During 1951 the plantation was acquired for experimental purposes and the distribution of die-back was studied during the course of a control experiment. In a block of 253 trees, 7105 local infections were diagnosed, an average of 28 per tree, and 101 of these had reached the trunk, some penetrating deeply and extending for several feet. Only fourteen young saplings were free from infection,

and seven trees (not included in the total) were so severely infected that they were felled. Many of the local infections in these trees were evidently established before the 1950-51 harvest but we are satisfied that most were a direct result of the harvest damage nine months before. Had the fungus been allowed to spread unchecked the history of this plantation would probably have repeated that of the one described above.

Possible control and remedial measures

Control measures should be based on reducing the quantity of inoculum and preventing the injuries which permit infection, combined with cutting out infected parts of trees in which the disease is not too far advanced.

One source of inoculum is the many dead trees, or dead branches on moribund trees, which occur in all areas. Plate 22, figs. 5 and 6, illustrate different stages of die-back in two plantations, both beyond remedial treatment. Unfortunately it has long been a deliberate policy to leave these dead trees standing to provide shade for young saplings. The first and most obvious control measure, therefore, is to remove and destroy dead material. We have found pycnosporos being extruded from the trunks of trees which have been dead for at least 9 months.

A second source of inoculum is the lesions on the trunks and branches of still living but infected trees. This is probably the most important source. Not only does infection in the upper branches of a tree give rise to secondary infections lower down on the trunk, spores being transported by water, but insects, birds and, most important, human beings probably also act as carriers. The clothing and person of a clove picker cannot fail to be liberally contaminated with spores. Inoculum on trees still bearing can be removed by cutting infected branches and excavating trunk lesions by standard methods of tree surgery. This process is made very much simpler by the fact that infected parts are clearly demarcated by a characteristic stain. Saplings from which infected areas were cut out, and the wounds protected by paint, more than a year ago, have demonstrated the practicability of this treatment.

Experimental treatment on a plantation scale has been applied to the second estate mentioned on p. 605. All diseased wood was cut away and the wounds treated with white-lead paint. Local African agricultural workers took readily to this task after short training, and the ladders and other equipment provided proved unnecessary as these men are accustomed to climb clove trees. In no treated tree has the fungus continued to spread, although, in a block of untreated trees, the disease is making obvious progress.

Many of the trees which were treated experimentally were so severely infected that, in practice, it is doubtful whether the treatment would be economically justified. Experience has shown, however, that large lesions, necessitating the removal of more than half the trunk over a distance of several feet, can be excised without having any appreciable effect on the vigour of the tree. Clove wood is extremely hard and tough and it seems unlikely that such treated trees will be

brought down by high winds. A concerted effort over a few years would probably reduce the incidence of *Cryptosporrella* die-back greatly, and might almost eliminate it. Young and uninfected trees can certainly, with adequate attention, be protected. This can only be done if harvesting methods are modified; the present ones, if persisted in, are certain to bring the industry to a premature end.

It is a pleasure to acknowledge the encouragement and practical assistance given us by Sir Vincent Glenday, who, at the time when this work was being done, was British Resident in Zanzibar. Our thanks are also due to Mr E. W. Mason, of the Imperial Mycological Institute, who has helped us with the taxonomy of *C. eugeniae*.

APPENDIX

Diagnosis

***Cryptosporrella eugeniae* Nutman & Roberts sp. nov.**

Species in truncis ramisque parasitica, lignum ad corticem rufo-brunneum colorans. *Pycnidia* in parte inferiore peridermii efformata, brunnea, depresso-conica, $\frac{1}{2}$ – $\frac{3}{4}$ mm. diam., per collum breve rumpentia, intus uni-locularia, subhyalina, strato prolifero labyrinthiformi vestita. *Sporophora* singula, ampulliformia hyalina, 8–12 μ longa, 2–3 μ lata. *Pycnosporae* ovoideae, continuae, hyalinae, 3·4–5 μ longae, 1·5 μ latae, in massa aurantiacae. *Perithecia* in primo singula, mox 2–10 vel plura in pustulas sublineares aggregata, globosa, brunnea, 500–900 μ diam., in collum aequilongum vel longius producta, intus ascis farcta. *Asci* obclavati vel fusiformes, saepe in aequilaterales, sessiles, apice leniter applanati, basi rotundati, tenuiter tunicati, 8-spori. *Paraphyses* nullae. *Ascosporae* sub-distichae vel oblique monostichae, oblongae, utrinque rotundatae, hyalinae, saepe 2-guttulatae sed nunquam septatae, 7–8 μ longae, 3–4 μ latae. *Hab.* In cortice *Eugeniae aromaticae*; in Insula Zanzibar, Africae or.; per annum, 1951. (F. J. Nutman & F. M. Roberts); specimen ut 44954 in Herb. I.M.I. depositum, typus. 4. iv. 1952

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* Files with the Department of Agriculture, Zanzibar.

DESCRIPTION OF PLATES 21 AND 22

PLATE 21

- Fig. 1*a*. Branch of clove tree infected with *C. eugeniae*. The top has been broken off by clove-pickers, and the upper shoots are dead. The lower shoots are still alive and healthy.
- Fig. 1*b*. The same branch slashed to show the stain caused by the fungus. A narrow band of as yet healthy tissue connects the healthy wood with the living twigs but is too narrow to be seen in the photograph.
- Fig. 2. The trunk of an 8-year-old clove tree which has been experimentally infected 8 months previously. The pycnidial necks protruding through the bark are clearly visible.
- Fig. 3. A young clove sapling about 18 years old, one main branch of which has been infected with *C. eugeniae* through the broken branch visible just above the slashed portion. The fungus has reached the main trunk, and the eventual death of the tree is inevitable.
- Fig. 4. A typical harvesting wound. The ragged break is obviously an efficient trap for spores. This branch is infected with *C. eugeniae* as can be seen by the protruding pycnidial necks around its base.

PLATE 22

- Fig. 5. A plantation of clove trees with very severe die-back. Most of the trees shown here are already infected in their main trunks, and remedial measures in such cases are rarely practicable.
- Fig. 6. The final stage of a plantation infected with die-back. In such a plantation all the trees not already dead bear only a few living branches connected with the roots by narrow bands of healthy tissues.
- Fig. 7. Example of 'slight' picking damage. It can be seen that the splintered wounds make efficient spore traps.
- Fig. 8. On the left a young clove sapling killed by *C. eugeniae* which attacked through a wound low down on the trunk. Such deaths have been confused with sudden-death.

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NUTMAN AND ROBERTS—*Acute die-back of clove trees in the Zanzibar Protectorate*



NUTMAN AND ROBERTS—*Acute die-back of clove trees in the Zanzibar Protectorate*

STUDIES IN THE BACTERIAL DIE-BACK AND CANKER DISEASE OF POPLAR

I. THE DISEASE AND ITS CAUSE

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(With Plate 23)

The bacterial die-back and canker of poplar is characterized by dark necrotic areas in the leaves, die-back accompanied by cracks in the bark of 1-4-year-old branches, and by cankers on the trunk and branches of different ages. The cankers may be 'closed' or 'open' and when moist exude slime containing bacteria.

The disease is caused by *Pseudomonas syringae* f. sp. *populea*, a new *forma specialis* closely resembling *Ps. syringae* in bacteriological characters but differing from it in parasitism. The association of the slime with the bacteria is essential for the production of typical lesions.

Inoculations with the pathogen alone, or in association with other organisms produce atypical symptoms.

The bacteria attack the cortical parenchyma and occasionally invade the xylem vessels.

INTRODUCTION

Delacroix (1906) described a bacterial canker in France which he attributed to *Micrococcus populi* Delacroix, but Regnier (1930) and Dufrenoy (1931) doubted this and the latter considered that an unidentified fungus caused the disease. The Imperial Forestry Institute, Oxford (1933-34) stated that a similar disease of poplars was one of the major silvicultural problems in Britain. The disease was described by Day & Peace (1934) who considered that frost was a contributory factor. Koning (1938) isolated *Pseudomonas rimae-faciens* Koning from a canker disease of poplars in Holland, and with it produced small atypical lesions. When, however, the slime was used as inoculum in spring, typical cankers were produced. Koning believed that there might be more than one organism in the cankers, which together were responsible for the disease. A bacterium identified as *Ps. syringae* van Hall was isolated by Lansade (1946) from poplars affected with die-back or bark-blight (les lésions de l'écorce) in France, and although he failed to reproduce typical symptoms by inoculating branches with it, Lansade believed that *Ps. syringae* could cause the disease in combination with certain environmental factors such as frost. In Holland ter Pelkwijk & Brink (1946) inoculated poplars with the bacteria and fungi isolated from the slimy exudate, separately and in combination, without result except that *Ps. rimae-faciens* gave rise to the atypical cankers previously

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described by Koning. Day (1948) expressed the opinion that frost was the primary cause of the disease and that the organisms associated with the lesions were secondary saprophytes, but the Forestry Commission (1948) considered that the disease was due to several bacteria acting together.

In 1945 one of us (W.J.D.) isolated from diseased poplar shoots a bacterium corresponding with Koning's *Ps. rimaefaciens* and with it and strains of *Ps. syringae* from lilac and Avocado pear inoculated young poplars in the open and under glass but with no definite results as the wounds healed over.

Early in June 1948 three young trees of *Populus eugenii* growing in the open were inoculated with the slime as Koning had done, with the result that two exhibited typical lesions in October 1948. From one of these a bacterium was recovered resembling *Pseudomonas rimaefaciens* which was handed over to the junior author (K.A.S.) who carried out a comprehensive investigation of the disease from October 1948 to July 1951. The account which follows is based on this investigation and a further paper concerning the production and properties of the slime will appear elsewhere.

SYMPTOMS

The disease is characterized by the presence of numerous cankers on the branches and by the dying back of many shoots. Two kinds of cankers can be recognized: one, a small (1-3 cm.) knotted excrescence is referred to here as a 'closed' or atypical canker (Pl. 23, fig. 3); the other, a larger (1-15 cm.) elongated perennial lesion with raised rim and exposed wood is designated as an 'open' or typical canker (Pl. 23, fig. 4). Both exude slime in spring during wet weather.

In spring many buds of 1-4-year-old shoots fail to open (Pl. 23, fig. 1) and cracks form at their bases from which slime exudes. Many tender young shoots are killed, leaves and petioles develop small brown spots or large black necrotic areas, and on the shoots bearing them, small (3 mm.) grey blisters arise. These open by cracks and exude slime (Pl. 23, fig. 2). Cracks exuding slime may also appear at nodes and the bases of laterals.

Affected shoots die in early summer, the leaves withering and falling off to leave dry, grey twigs on which fructifications of *Nectria*, *Fusarium* and *Cytospora* appear: these fungi however were shown to be non-pathogenic. *Populus eugenii* and *P. trichocarpa* are very susceptible to attack and exhibit a preponderance of 'open' cankers, while on resistant species such as *P. serotina* and *P. marilandica* 'closed' cankers are more frequent.

MATERIALS AND METHODS

Diseased branches were collected from East Anglian plantations and a large number of healthy sets of various species were provided by Mr T. R. Peace of the Forestry Commission for infection experiments. These were planted either in the open or in pots in the greenhouse.

Twigs and branches were fixed in formalin-acetic-alcohol, dehydrated in tertiary

butyl alcohol and embedded in celloidin. Transverse and longitudinal sections were cut, 10, 15 and 30 μ thick, and stained with safranin, fast green and orange G.

For leaf and shoot experiments, potted plants, after inoculation, were kept for a week in shallow trays containing water and covered with metal cylinders with glass tops. Shoots were inoculated through wounds made by the point of a scalpel, the wounds being either wrapped round with moist cotton-wool and lead foil or left uncovered. Leaves were inoculated either through wounds or by applying diluted slime to the lower surface with a camel-hair brush.

Controls inoculated with sterile water accompanied each set of experiments but produced no pathogenic symptoms.

ISOLATION

Isolations were made monthly but mainly in May from the following diseased material. The spots on the leaves were cut out and after rinsing in a 0.2% solution of sodium hypochlorite for 2 min. were washed twice in sterile water. The pieces bearing the spots were then teased apart in water with flamed scalpels and left to diffuse for 4–6 hr. Plates were streaked from the resulting liquid.

The twigs were cut into pieces 5–7 in. long and after washing under the tap were placed on sterile cotton-wool saturated with sterile water in enamel dishes. After 24 hr. slime exuded from the cracks and from lenticels. Slime so obtained was designated 'natural' slime, and after suspension in sterile water at 25°C. for 2–6 hr. was streaked out on to meat-infusion agar plates.

Isolations were also made from diseased tissue beneath cracks and from both types of cankers. Small pieces were cut out, dipped in 95% alcohol for a moment and after flaming were transferred to Petri dishes containing about 20 ml. sterile tap water. The tissues were teased apart and left for 8–16 hr. after which the liquid was streaked on to meat-infusion agar.

Young shoots bearing small blisters but no cracks were cut into pieces 2 in. long, which after washing in running water and immersing for 5 min. in a 0.2% sodium hypochlorite solution followed by two rinses in sterile tap water, were laid on moist sterile cotton-wool in glass dishes. The blisters were then punctured with the tip of a flamed scalpel. Slime exuded from the punctures within 24 hr. and was collected aseptically, suspended in sterile water and plated out. Slime obtained in this way was designated 'pure' slime.

Suspensions were also made from leaves bearing discoloured spots thought to be early stages of infection. The spots were cut out and after treatment with sodium hypochlorite and rinsing in water were placed in sterile water for some hours prior to plating out.

Bacterial colonies predominated on all the plates but a few fungal growths also appeared which were later identified as *Fusarium lateritium* Nees.

The majority of bacterial colonies were white, and surrounded by a greenish fluorescent zone indicating the genus *Pseudomonas*. A few yellow colonies also

appeared on some of the plates. On examining the green-fluorescent colonies it soon became evident that most of them corresponded closely with *Ps. syringae* van Hall, not only in their cultural characters but also in pathogenicity indicated by inoculating lemons, whereby dark necrotic depressed areas resulted similar to those (citrus blast) caused by *Ps. syringae*.

Eighteen of the isolations thus obtained together with the one isolated in 1945 (W. J. D.) were transferred monthly to slopes of either meat-infusion agar or pectin-bactotryptone agar (pectin 0.4%, Difco bactotryptone 1%, agar 1.5%) and were used for inoculation.

INOCULATION EXPERIMENTS

The following inoculations were made in young trees of *Populus trichocarpa*, *P. eugenii*, *P. alba*, *P. fastigiata*, *P. regenerosa* and *P. carrieri* both in the open and under glass.

I. Inoculations with 'natural' slime (Pl. 23, fig. 5)

Shoot inoculations

128 inoculations in all were made between the end of April and the end of August 1949 and during the same period in 1950 of which the following may be mentioned. Of 90 made in 1949, 14 resulted in 'open' cankers by October, 53 produced 'closed' cankers and 8 shoots died back. In the following year the 53 'closed' cankers developed into the 'open' type. 20 inoculations made in June-July 1949 produced 2 'closed' cankers and 4 died back, while 13 inoculations in August 1950 failed to cause any infection.

Within 2-3 weeks of inoculation infection was revealed by the darkened and depressed epidermis in and around which cracks developed. The progress of infection appeared to cease in late summer but was renewed the following season when slime exuded from the cracks. *Populus trichocarpa* was the most susceptible of the species inoculated and *P. fastigiata* the most resistant.

A bacterium corresponding with *Pseudomonas syringae* was recovered in May 1950 from cankered shoots of *Populus trichocarpa* inoculated a year previously.

Lenticel inoculations

All attempts to bring about infection via lenticels failed.

Leaf inoculations

Several leaves of young trees grown under glass inoculated in spring either by wounding or by applying diluted slime to the lower surface in 1949, resulted in either small, brown spots about 0.5 cm. across, or more often, large black necrotic areas within 2 weeks. Similar results were obtained in 1950. Late in summer cracks appeared in the brown areas the centres of which fell out leaving a 'shot-hole' effect. The bacteria could not be recovered from these lesions in August 1949, but in 1950 the organism was re-isolated from the necrotic areas within 3 weeks (May) of inoculation by which time they had reached maximum size.

II. *Inoculations with 'pure' slime* (Pl. 23, fig. 6)

Of 4 shoots of *Populus trichocarpa* grown under glass and inoculated in May 1949, 3 developed 'closed' cankers and one failed to become infected. In May 1950, 10 inoculations were made in the shoots of *P. eugenii* and 10 in *P. trichocarpa*, which resulted in the formation of 3 'open' and 4 'closed' cankers in the former, and 2 'open' and 8 'closed' cankers in the latter; a total of 20 cankers out of 24 inoculations.

III. *Inoculations with a pure culture of the bacterium, suspended in water*
(Pl. 23, fig. 7)

From March to December 1949, 8-16 inoculations were made in shoots of each of the following species: *P. trichocarpa*, *P. eugenii*, and *P. carrierii*. In all 240 inoculations were made. These resulted in 84 temporary lesions which healed over the same season and 156 which showed no sign of infection.

Leaves inoculated in 1949 and in 1950 in the same way as in § I (above), invariably developed small brown spots which never became aggressive. The organism could be recovered from the spots within 3 weeks of inoculation but not afterwards.

IV. *Inoculations with sterile filtered slime*

As the fresh 'natural' slime was very viscous and difficult to handle it was diluted with 10-20% of its volume of sterile water, and centrifuged at 3000 r.p.m. for 30-60 min. The supernatant liquid passed through a Seitz filter was found to be sterile. No infection was obtained by inoculating this liquid into either shoots or leaves.

V. *Inoculations with a pure culture of the bacterium suspended in sterile filtered slime* (Pl. 23, fig. 8)

Leaf inoculations produced spots and necrotic areas similar to those caused by inoculating 'natural' slime (§ I above). When, however, shoots were inoculated, only 35% resulted in typical symptoms as indicated in Table I. The majority of the

TABLE I. *Inoculations with the bacterium suspended in sterile slime filtrate into shoots*

Date of inoculation	Species of <i>Populus</i>	No. of inoculations	Typical symptoms				Atypical symptoms	No infection
			Die-back	Closed cankers	Open cankers	Total		
4. v. 49.	<i>P. trichocarpa</i>	8	2	—	1	3	5	—
	<i>P. carrierii</i>	8	3	1	—	4	4	1
26. v. 49.	<i>P. trichocarpa</i>	4	—	1	1	2	2	—
	<i>P. carrierii</i>	3	—	1	—	1	2	—
5. v. 50.	<i>P. trichocarpa</i>	12	1	2	1	4	5	3
	<i>P. eugenii</i>	6	1	1	—	2	1	3
21. v. 50.	<i>P. trichocarpa</i>	8	1	2	—	3	5	—
	<i>Populus sp.</i>	8	—	1	—	1	6	1
	Totals	57	8	9	3	20	30	8

inoculations gave rise to temporary lesions resembling those produced by the bacterium when inoculated alone (§ III above). The cankers took longer to develop than those produced by inoculating 'pure' slime (§ II above), from which it might be argued that in diluting and centrifuging the slime, some indispensable constituent had been so weakened as to retard the rate of invasion in the majority of inoculations, thereby enabling the host to overcome infection.

VI. *Inoculations with the isolated bacterium in combination with other bacteria and various fungi isolated from the lesions (cankers, cracks, dead shoots)*

These resulted in temporary infections similar to those produced by inoculating the bacterium alone (§ III above).

VII. *Cross-inoculations*

As the poplar bacterium closely resembled *Pseudomonas syringae*, a series of inoculations was made with the two organisms into different hosts. Poplar (shoots), lilac (shoots), lemons, tangerines and pears were used for this purpose. All the hosts were infected except the lignified branches of poplar in which both organisms caused temporary lesions only. In the four other hosts the lesions produced by *Ps. syringae* were more severe than those caused by the poplar bacterium indicating a distinct difference in pathogenicity between the two organisms. When, however, the poplar bacterium was introduced together with filtered slime, the lesions formed in lilac, lemon, tangerine and pear were similar to those brought about by *Ps. syringae*.

In lignified branches of poplar, the poplar bacterium inoculated together with slime caused a much more severe infection than *Ps. syringae*.

A consideration of the information obtained during two seasons' infection experiments led to the following conclusions:

(1) The disease was exactly reproduced by the inoculation of leaves and shoots in spring with 'natural' slime, or with a bacterium isolated from the slime together with filtered sterile slime.

(2) Temporary lesions were produced by inoculating the bacterium alone.

(3) No infection resulted from the inoculation of filtered sterile slime.

(4) There was therefore an intimate connexion between the isolated bacterium and the slime, both being necessary for the production of typical symptoms.

(5) Other organisms present in the slime played no part in the production of typical symptoms.

(6) Leaves could be infected through the stomata and wounds, but the shoots only through wounds.

(7) Infection took place in spring and early summer and at no other time.

(8) The bacterium isolated from the slime and from the lesions resembled *Ps. syringae* van Hall but differed from it in pathogenicity.

IDENTITY OF THE POPLAR BACTERIUM

In its morphological and cultural characters the bacterium differs from *Ps. syringae* van Hall in forming a little acid in maltose (in a peptone-free medium of pH 7.0, with bromthymol blue as indicator) and in being strictly aerobic.

Pathogenically it is less virulent when inoculated into lilac, citrus or pear, unless introduced with the slime (filtered) produced in the poplar lesions; and whereas *Ps. syringae* passes the summer in the leaves and inflorescences of its hosts and the winter in dormant buds, the poplar bacterium is perennial in branches but rapidly dies out in leaves and green shoots. These differences are not considered sufficiently important to justify the creation of a new species; they are however, indicative of a special form of *Ps. syringae* and the name *Ps. syringae* f. sp. *populea* is proposed for it.

It is further considered to be synonymous with *Ps. rimaefaciens* Koning.

PATHOLOGICAL EFFECTS

The periderm is not directly invaded but is mechanically ruptured and produces thick layers surrounding the 'open' cankers. The cortical parenchyma is severely attacked and in it cavities and blisters are formed full of slime. The cavities are subsequently cut off by periderm. The bast fibres are not attacked but are surrounded by heavily infected layers of cells in the intact bark. The cambium may be attacked before or after the formation of new wood and is killed either in several small adjacent spots or over a large continuous area, whereby 'closed' or 'open' cankers are formed respectively. In the former the cambium continues to produce wood and phloem, and a callus partially heals the cleavages which become narrower but do not close (Pl. 23, figs. 3 and 9).

In the following season new rents appear at more points followed by further attempts at healing over. In 'open' cankers callus is formed at the edges, but is never sufficient to heal the wounds which remain open (Pl. 23, figs. 4 and 10).

The wood is not generally attacked but a few of the vessels of both primary and secondary xylem, or near a leaf or branch trace, may be invaded and rather irregular annual rings are sometimes formed. A brick-red stain is formed in the wood around cracks and cankers, leaf and branch traces and the medulla. The medullary rays are invaded and are very liable to infection near leaf or branch traces where cleavages in the wood occur.

DISSEMINATION OF THE DISEASE

The disease is disseminated from place to place most probably by planting slightly infected sets in which the disease develops rapidly during spring and early summer. This may be brought about in several ways. The slime exuding from the cracks is washed along the shoots by rain to cause further infection through wounds and possibly through leaf and bud-scale scars. The bacteria beneath the cracks spread in the cortex upwards and downwards for considerable distances and wind-blown

raindrops contaminated with slime may cause infection of young leaves through the stomata.

The most interesting feature of the disease brought out by the present investigation is the intimate association between bacterium and slime both of which are essential for producing typical symptoms. It may be that a similar association exists in the slime disease of Cocksfoot grass (*Dactylis glomerata*) which hitherto has been reproduced only by inoculation of the natural slime (Dowson & d'Oliveira, 1935).

We wish to acknowledge our grateful thanks to Prof. F. T. Brooks, C.B.E., F.R.S., for his helpful criticism and unfailing interest throughout these investigations.

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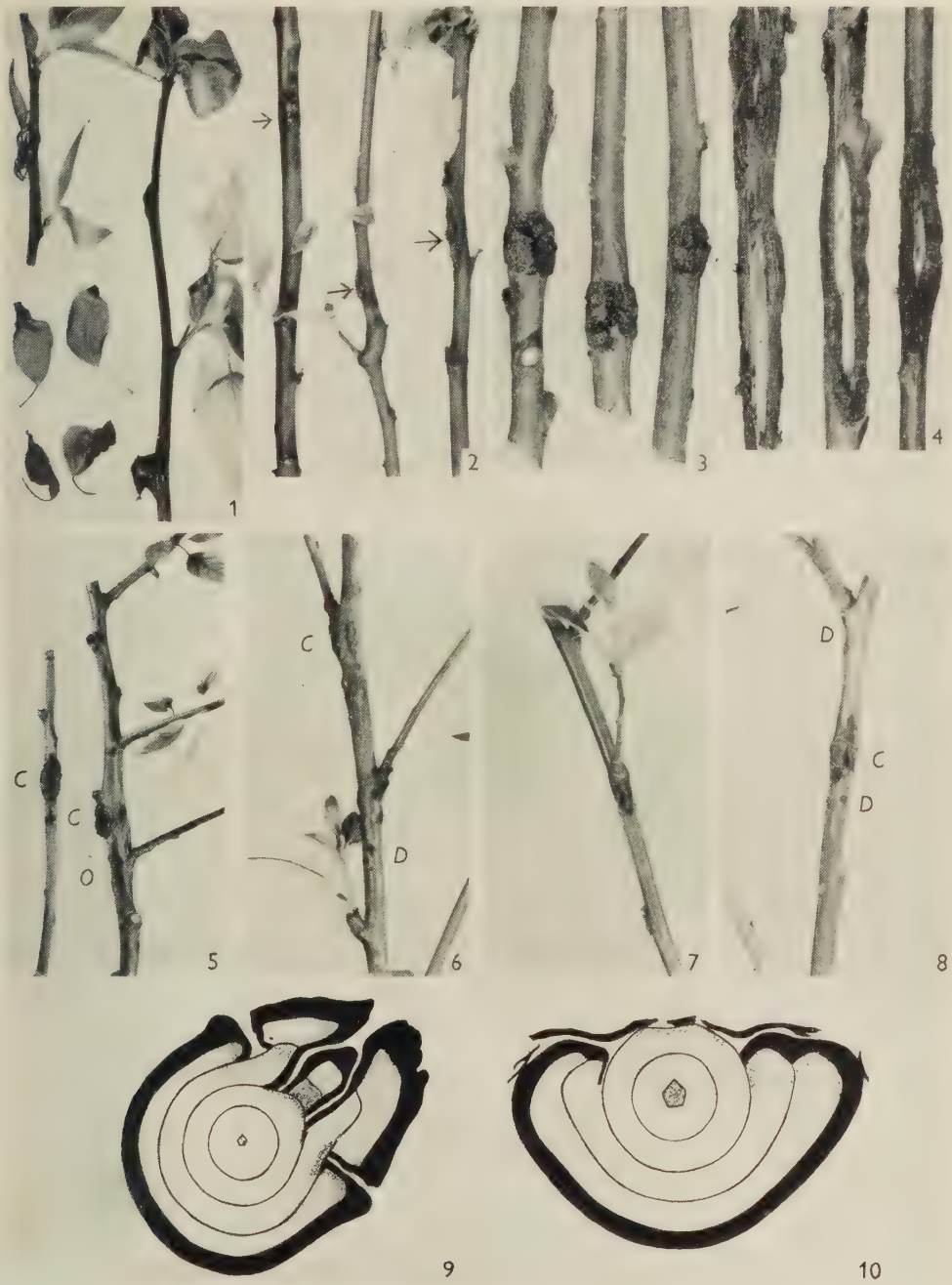
EXPLANATION OF PLATE 23

- Fig. 1. Twigs of *Populus trichocarpa* showing leaf and green shoot blight ($\times \frac{1}{4}$).
- Fig. 2. Twigs of *P. trichocarpa* showing the cracks and exudation of bacterial slime ($\times \frac{1}{4}$).
- Fig. 3. Branches of *Populus* sp. showing closed cankers ($\times \frac{1}{4}$).
- Fig. 4. Branches of *Populus* sp. showing open cankers ($\times \frac{1}{4}$).
- Figs. 5, 6, 7 and 8. Inoculations in *P. trichocarpa* photographed after a year; fig. 5, with natural slime; fig. 6, with pure slime; fig. 7 with a pure culture of *Pseudomonas syringae* f. sp. *populea*, and fig. 8 with the organism suspended in sterile slime filtrate ($\times \frac{1}{4}$).

C = 'closed' canker, O = 'open' canker, D = new cracks.

- Fig. 9. A diagrammatic representation of a 'closed' canker, stippled area = reddish discoloration ($\times 2$).
- Fig. 10. A diagrammatic representation of an 'open' canker, stippled area = reddish discoloration ($\times 2$).

(Received 15 February 1952)



SABET AND DOWSON—Studies in the bacterial die-back and canker disease of poplar

OBITUARY NOTICE

PROFESSOR F. T. BROOKS, C.B.E., F.R.S.

Frederick Tom Brooks was born at Wells, Somerset on 17 December 1882, the youngest of a family of three girls and two boys. He was educated at Sexey's School, Bruton, of which he became a governor in later years and it was in and around Bruton during his school days that Brooks first displayed a great interest in field botany. At the relatively early age of fifteen he left school to enter a teachers' training college at Bristol and it was here that his inherent powers of learning and teaching became evident. In every examination he entered for, the result was always the same, a first class; in 1902 he was awarded an exhibition at Emmanuel College, Cambridge, where he gained first class honours in both parts of the Natural Sciences Tripos, making botany his main subject. As a result of his success in the Part II examination Brooks was awarded the Frank Smart Studentship in botany given by Gonville and Caius College which entailed joining that college for the two-year period of the studentship. During this time Brooks came into close contact with his professor, the late Marshall Ward, who was so impressed with his ability and capacity for hard work that he appointed him junior demonstrator in botany for the maximum period of five years.

After Marshall Ward's early death in 1906, Brooks at the age of 24 and still a demonstrator, carried on the teaching of mycology and plant pathology bereft of the guiding hand of the one person who had such faith in him, a faith fully justified for the next forty years. Brooks initiated a two-year course for these subjects, dealing with the Phycomycetes and Ascomycetes in one year followed by the Basidiomycetes the next year.

In 1913 he was seconded to the then Federated Malay States to investigate certain diseases of rubber trees (*Hevea brasiliensis*), the accounts of which were subsequently published in these *Annals* for the years 1916 and 1917. He just managed to get back to England before the 1914 war broke out, and, as a keen patriot and former member of the then Cambridge University Rifle Volunteers, he endeavoured to enlist in the armed forces but was rejected for active service on account of his sight. Determined to do whatever he could in the national interest he made a survey of the spread of potato blight and on the formation of the Food Production Department became its plant pathologist, investigating among other things the moulds appearing on chilled meat delayed in transit from overseas, and the fermentation of maize by a bacterium, known as the 'XY bacillus' for the production of acetone. This was extremely important work, as acetone was in great demand as a solvent in the production of explosives and at the height of the submarine campaign the quantity of explosives for our armed forces in Flanders depended entirely on the number of maize-ships running the blockade.

In 1919 Brooks returned to the University which had been practically closed during hostilities and was made a lecturer, partly to replace R. P. Gregory who had died the year before from influenza during the epidemic of that year. Gregory's teaching had been mainly concerned with the algae and genetics, but his lecture notes were not available and Brooks had to set about constructing the two courses anew. This entailed much additional work, and at one period during the next few years he was lecturing and demonstrating four times a week in as many different subjects. Despite this, he carried out lengthy investigations on silver-leaf disease, probably his best-known work, partly in co-operation with former students. Many other diseases of cultivated plants were investigated from this time onwards and also some interesting and little-known fungi which turned up in curious and unexpected places, such as *Eremascus albus* in a tin of mustard which had been converted into a solid white block. Some of these researches he never lived to complete: thus his prolonged study of the rust of brome grasses in which he had been engaged for the past twenty years was

never finished and also his work on an interesting wilt of privet, discovered on a local field excursion and found to be due to a species of *Glomerella*.

In 1931 Brooks was made a Fellow of his college, Emmanuel, and was also elected a Fellow of the Royal Society, both well-merited distinctions. In the same year a generous gift from the Rockefeller Foundation to various departments of the University made it possible to add a considerable extension to the Botany School and also to acquire some three acres of land on the University Farm for an experimental Field Station. On this Brooks had an extensive glass-house and a small field laboratory built, and planted a selection of economic and ornamental trees, shrubs and herbaceous plants for the investigation of their various diseases.

The Rockefeller bequest also enabled him to initiate a new course of lectures and laboratory work on bacteria and bacterial plant diseases which entailed additional staff and the creation of a readership in mycology which he held for the next five years, when he was elected to the Chair of Botany on the retirement of Sir Albert Seward. In 1947 Brooks was made a C.B.E., an honour which was much appreciated, and in 1948 having reached the age of sixty-five he retired from the professorship, but not from the Botany School in which he carried on his research work, dealt with his voluminous correspondence and was constantly sought after for advice and guidance by students and colleagues alike.

Although below the average height, Brooks was sturdily built and a keen participant in various sports. In summer he played tennis and squash racquets, went for a swim in the Cam most mornings throughout the year, running all the way there and back from his house in Tenison Avenue. On botanical excursions he would walk most others off their feet and invariably insisted upon taking the students during the 'long vac.' across the roughest part of Chippenham Fen. It is possible that the fatal heart attack which laid him low had its origin in these very vigorous pursuits, especially squash racquets, and a serious abdominal operation in 1940 compelled him to give up most of them. He made a remarkable recovery and it was not until ten years later that a severe attack of bronchitis in the spring left him with a much weakened heart necessitating a complete rest that summer which proved most irksome to such an energetic individual. During the following year he seemed to recover his old vigour but a second attack of bronchitis at Christmas 1951 left him physically very weak. Even so, his indomitable spirit and profound sense of duty impelled him to carry on although he could no longer walk very far and had to resort to taxis to take him to the Botany School; on occasions he even went up to London to attend some meeting. On the day he died I had spent some time with him and on leaving he handed me a £1 note to buy four 5s. stamp books which I was to bring him without fail the next day. The stamp books were bought that afternoon; but he never received them for the end came very suddenly as he was preparing for bed that evening, the 11th of March.

All who knew him admired his downright sterling character and one felt that one could place the utmost reliance in anything he said or did. Ever a good correspondent, letters to him received a prompt reply and during both world wars when mails were uncertain, the first intimation of a letter getting safely home was the arrival of his reply. His days were sharply divided into two phases; one in which he was the rather stern and dignified Professor in the Botany School and the other when he was the genial host and enthusiastic gardener at home. He was held in some awe by most of the students and even some of his colleagues felt a little perturbed on finding on their benches a note with 'can I see you, please' in his very characteristic spidery handwriting. He never minced matters or beat about the bush but came straight to the point and if it was your slip-up, you were certainly made to realize it, but he was always fair and just and never resentful. Apart from his own work or that connected with the department, Brooks continually had in mind the interests of the students, particularly those whom he had sent out into the world as mycologists or plant pathologists and who wrote to him regularly.

His manifold activities could only have been achieved by the possession of a most orderly

and logical mind. His work bench and writing table were always extremely neat and tidy; everything had its proper place and was put back there after use. There was a recognized time for everything, interviews, laboratory work, reading and correspondence. Brooks's correspondence was enormous and increased rather than diminished as time went on, for he was closely associated with many institutions such as the Agricultural Research Council, the Forestry Commission and a number of governing bodies, the regular meetings of which absorbed a great deal of his time and energy. Nevertheless, during the last three years he found time among many other things to rewrite entirely and enlarge considerably his book, *Plant Diseases*, which is now in the press.

Brooks's university lectures and public addresses were most carefully prepared and he would put into one hour's discourse an amount of information and comment for which many others would require a good deal longer. During the forty years he taught at Cambridge many senior workers, apart from his research students, came to spend some months and even a year with him, studying some particular problem in mycology or plant pathology. All in all, Brooks was responsible for the training of close on one hundred specialists in these branches of botany, a wonderful achievement for a single individual and a very practical illustration of his powers of stimulating and inspiring young people in the study of the fungi from every aspect.

He died in harness, as he would have wished, mourned by many who will ever remember his hearty laugh and revere his resolute character and transparent honesty.

W. J. DOWSON.

REVIEWS

The Origin, Variation, Immunity and Breeding of Cultivated Plants. Selected writings of N. I. VAVILOV, translated from the Russian by K. STARR CHESTER. *Chronica Botanica*, Vol. 13, Nos. 1-6. Pp. viii + 366. Price \$7.50. Waltham, Mass.: Chronica Botanica Co.; London: Dawson.

Nikolai Ivanovich Vavilov must be almost as well known a name among biologists as Morgan or even Mendel himself. He is familiar as the leading Russian geneticist; but his is chiefly the melancholy fame of one who fought for his science in a great controversy and paid for his failure with his life. His contributions to genetics—indeed we may say to plant science as a whole—have been known to most only in outline, largely because much of his publication was in the Russian language, which few of us can read. Now, thanks to Dr Starr Chester and *Chronica Botanica*, we have available to us, for our own study and appraisal, five essays written by Vavilov and taken from the 2500-page Russian symposium on *The Scientific Bases of Plant Breeding*, which was published under Vavilov's editorship in 1935, and which, as Dr Chester says in his preface, marked the climax of his career. Though others have worked on the translation of parts of these essays, it is to Dr Chester's effort that we owe this volume. I cannot, of course, speak of the accuracy of the translation, but the care and skill of writing which have gone into it will be obvious to all. We are indeed indebted to Dr Chester for the labour which has added a unique volume to our library of plant science. And we are indebted to *Chronica Botanica* for its production, and not the least for the production or reproduction of the cuts of primitive agricultural instruments and methods which appear on the title pages and elsewhere.

The five essays comprise a short introduction on 'Plant Breeding as a Science'; three rather longer ones of about fifty pages each on the 'Phytogeographic Basis of Plant Breeding', 'The Law of Homologous Series in the Inheritance of Variability' and the 'Study of Immunity of Plants from Infectious Diseases' respectively; and a final discussion of the 'Scientific Bases of Wheat Breeding', which occupies some one hundred and forty pages and to which is appended a twenty-page bibliography taken from the world literature of wheat breeding and genetics.

This last is indeed a monumental work. We find within it discussions of the constitution, variation and potentials of wheat; of its biology, ecology, development and genetics; of the parentage of the great varieties of Europe, America and Australia; of the characteristics which an ideal wheat must possess and the ways in which we should set about breeding for them; of the present state of wheat breeding in the many lands where it is practised; and of the behaviour and possibilities of crosses between wheat and its relatives, *Secale*, *Aegilops*, *Agropyrum* and *Haynaldia*. We have here a veritable mine of information to which may turn all who are interested in wheat whether as an object of genetical and taxonomical study or as one of the major crop plants of the world. One table, dealing with the distribution of variation among the three chromosome groups of the genus, itself covers nearly eight pages. Another seven pages are devoted to summarizing our knowledge of the genetical control of this variation in the 42- and 28-chromosome wheats; the pedigrees of fifty-two varieties are set out; and forty-six basic requirements of a wheat variety are listed. There can be few aspects of wheat breeding which have escaped Vavilov's attention, and the factual information which he presents is continually illuminated by his shrewd comment.

The three essays on phytogeography, homologous variation and disease immunity are less concerned with individual species and more with principles. This is not to say that the factual bases of the principles are neglected: indeed, there is a wealth of tabular matter including

lists of the plants, classified according to their uses, which he regards, on the basis of their variation, as having originated in the eight great centres he recognizes as a result of the immense programme of plant exploration and collection he organized. This list will be a revelation to many of us, for the immense variety of plants for which man has found a use, to mention nothing else. Even so the interest in these essays must centre on the principles they seek to develop, and for which Vavilov is famous, in genetical circles at least: the principles that the centre of maximum variation is the centre of origin, that related species show homologous variation, and that there is a genetic balance between host and parasite. These principles and their implications have been much discussed since they were enunciated. Few would now agree that the centre of variation is necessarily the centre of origin, or that the law of homologous variation necessarily implies a common ancestry of the genes whose changes produce the variants, though of course such common ancestry is one great cause of homologous variation. But to view the principles in this way would be to miss the value they held for Vavilov and his fellow applied geneticists, and indeed to miss the whole significance of Vavilov's point of view. These essays make clear that, to him, the principles were generalizations of his vast experience: empirical statements, having genetical and evolutionary implications which it might be interesting and instructive to discuss, but whose value was essentially pragmatic in that they led him to a clearer and broader understanding of the possibilities of his crop plants, of what he could aim at producing by plant breeding, and of where he could most usefully seek for the raw materials for his plant breeding operations. Viewed in this way, his principles have stood the test of time. His insistence on wide collections of wild forms and relatives as well as cultivated is universally shared, as providing the only full basis for plant breeding programmes, and his collecting expeditions have been copied even to the centres from which the collections have been made, though still too infrequently and on too small a scale to give us the gene reservoirs that we need. His approach to the taxonomy of his materials, coloured as it is by the genetics he developed and applied, is one which more and more have come to value.

To judge Vavilov on any narrow interpretation of genetics would be wholly false and misleading, and indeed it is the final irony of his fate that he was condemned for his acceptance and defence of a science which his critics perversely represented as narrow and divorced from practical affairs, yet which he had done so much to broaden and to apply. He was an applied biologist in the fullest sense of the words: a man who sought to apply his biology to the service of man, in the all-important field of food supply. In doing so he used genetics as a tool, the chief tool maybe, but still only one tool of many. And, as always happens, genetics itself became the richer and more powerful for its use.

In the first essay, which although the shortest is the key to all the rest, we have Vavilov's view of plant breeding set out before us. 'Plant breeding', he opens, 'is a science, an art and a branch of agriculture', which is to be related 'not only to genetics but also to systematics, geography of plants, biology of flowering, cytology, ecology, biochemistry, physiology and technology.' He is using genetics here in a narrower sense than many of us, in building on his work, would now think desirable; but, even so, his breadth of vision emerges clearly. The whole volume speaks of the singleness of purpose in pursuing the goal of better crops, of the driving energy with which it was pursued and of the willingness to enlist any science in the pursuit. We see him, too, relating his work to the needs of his country, and here and there catch a suggestion of pride in the system which had given him the opportunity to work on such a scale, and which was finally to abandon him.

These essays were written at a time when the great controversy had already begun, and it may be that Vavilov's desire to set down a full statement of his views was sharpened thereby. The deepening shadows are perhaps detectable in the frequent references to Lysenko's work on vernalization which to Vavilov was still another, and possibly valuable, aid in the pursuit of his goal. He does not reject Lysenko's findings. Rather he wishes to incorporate them into the plant breeder's armoury, to let them do what they can towards the increase of

production. Possibly Vavilov was too broad-minded for his own personal good: his readers can form their own opinion. But on one point there will be little disagreement. This volume is a worthy memorial to a great biologist, whom geneticists are honoured to have numbered amongst them.

KENNETH MATHER

General Biology. By PERRY D. STRAUSBAUGH and BERNAL R. WEIMER. Pp. 813. 3rd edition. London: Chapman and Hall Ltd. 1952. 48s.

This book approaches more nearly to the usual type of English text-book than do most of the recent American text-books on biology. This may have been one reason why it appealed more strongly to the reviewer than have other American books. But there are also other reasons; the presentation is logical, the illustrations are good—there are twenty beautifully produced coloured diagrams—the type is clear, the paper is excellent and the book is a beautifully finished product of author, printer and publisher. There is also ample evidence of an admirable effort to bring all sections up to date, and consequently of very wide reading on the part of the authors; this being so it is a great pity that in the text references to modern literature are very scanty and there is none at the ends of the chapters.

The text develops the study of plants and animals simultaneously, the principles of their metabolism, growth, reproduction, heredity and taxonomy are dealt with side by side and then the plant and animal kingdoms are separately surveyed, the whole being built up to an admirably balanced text-book.

The book is especially suitable for students at the Higher Certificate, 1st M.B. or Intermediate level, but at 48s. few will be able to afford it in addition to the English text-books generally recommended at British Universities.

I. THOMAS

Man and the Animal World. By BERNAL R. WEIMAR. Pp. 569. New York: J. Wiley and Sons; London: Chapman and Hall Ltd. 40s. net.

‘This book attempts to treat the organisms of the animal world as dynamic living entities, manifesting in various ways the fundamental life phenomena common to all living animals.’ This is the avowed aim of the author, and in this he has very largely succeeded.

The book begins with a study of man as the best-known animal and thereby employs what the author believes to be the logical approach to the study of elementary physiology and morphology. This is no new method, but in other ways the book is different from the ordinary run of English text-books; it is written in such a way that it maintains a strong human appeal throughout. Many of the examples and illustrations are of abnormal human beings and animals, and although this is deliberate it is probably overdone because some of the chapters are reminiscent of a medical or veterinary case-book. There are 315 drawings and photographs and sixteen coloured plates; most of the drawings are purposely diagrammatic and some are crude, but on the whole they serve their purpose.

The book is written primarily for undergraduate students, and the presentation is such that it should be readily followed and understood by students with little or no training in biology. Dr Weimar has been completely successful in making the chapters on elementary genetics and taxonomy clear and interesting but, as with all text-books, some chapters are out of date before the printer’s ink is dry. This is to a large extent inevitable when dealing with a subject such as the use of insecticides for pest control, but there is surely no excuse for saying of a number of Diptera ‘The only means of fighting these flies is by repellents.’

The treatment of hormones and vitamins is clear and succinct, and there is an excellent chapter introducing the student to the subject of the animal in relation to its environment, in which wild-life conservation is briefly discussed. All the chapters include pertinent practical everyday facts which tend to whet the appetite. Every technical term used is

clearly defined and its derivation is given, but this being so, English gorges will rise even more at the use of 'larvas' for 'larvae' and 'cheliceras' for 'chelicerae'.

Viewed through British eyes this book may have its faults, but it is a challenge to writers of text-books of zoology in the traditional style and with many students it will probably succeed in stimulating a genuine interest in zoology where other books will fail.

I. THOMAS

Biology: its Human Implications. By GARRETT HARDIN. Second edition. Pp. 720 with 250 illustrations in text. San Francisco: W. H. Freeman and Co. 1952. \$5.00; 42s. 6d.

This substantial and freshly illustrated text-book is one in which human physiology occupies the central position, with all other branches of pure and applied biology in peripheral relation to it. The modest claim made for the book is that it contains 'what a student should learn in a college course in biology if he will never again be exposed to formal instruction in the subject', and indeed, the non-medical students of California University who go forth equipped for life with so much sound teaching on the functioning of the human body and of biology as a whole are very much to be envied. In this country such a course of instruction does not fit in with our examination system, and perhaps it would be best to say that the book integrates the biology for 1st M.B. and is a good introduction to pre-clinical work at the university. But it is also a good family reference book on biology, where there are intelligent sons and daughters in their 'teens, and parents who sometimes want to look up what the doctor does not tell them on the causes of diseases, and the facts and theories of evolution, chromosome entanglements, enzymes and the rest. An attractive feature of the book is the careful definition of biological terms, introduced one by one in bold type in the text, and easily found for reference. The many diagrams are plain and clear; they are the work of a good artist, who really understands the job of illustrating a teaching text.

An introductory section gives a general view of the science of biology and of scientific method, and explains the cellular and molecular basis of living phenomena. Then, under 'The Measure of Man', there is a closely packed and most informative introduction to human physiology, dealing with the muscular, respiratory, digestive and circulatory systems, disease, blood groups, nerves, the senses, and human reproduction. Under 'Heredity' there are sections on the cellular details of reproduction, the particulate nature of inheritance, genes, and the problems of gene action. Under 'Evolution and the Living World', come the theory of evolution, the names and sizes of organisms, bacteria, fungi, algae, plants, invertebrate animals, the arthropoda and the vertebrates. Finally, under 'The Web of Life', there is more speculative matter on the nature and origin of life, energy and population problems and the future evolution of man.

The author's intention has been to *teach*, and this he does responsibly, forgoing temptations to stray off into controversy, presenting the weight of evidence for present beliefs and orthodoxies, but throughout encouraging the student to think independently and for himself. The book is the product of very wide reading, to which full references are given, and it has been no small achievement to present the substance of all this matter, in a fresh, crisp and logical style that could hold at least one reader's interest, without flagging, over more than 700 pages. There is much philosophy in the book, and the group of university students whose opinion I sought on it, liked it for its logical positivism.

We have to be abstemious in these days in the purchase of books, especially where it means spending dollars, but this is one that librarians of secondary and grammar schools, technical institutes and training colleges, as well as university departments, can fairly be recommended to see and consider for purchase.

E. C. LARGE

General Genetics. By A. M. SRB and R. D. OWEN. Pp. 571 with 182 illustrations in text. San Francisco: Freeman and Co. 1952. 47s.

This is an excellent introduction to genetics, well balanced and up to date. The first seventeen chapters dealing with 'classical Genetics' and those fields of genetics usually found in text-books show in places a most refreshing approach and they are well integrated. Methods are described in sufficient detail and the relevance of the conclusions drawn is frequently pointed out.

Chapters 18-21 on Genes and Development, (The Nature of) Genes, Population Genetics and Genetics and Evolution are lucid expositions at an elementary level of these modern branches of the subject and as such they are acceptable. Three concluding chapters on Plant Breeding, Animal Production and 'Genetics and the Welfare of the Human Individual' point to the main applications of genetical knowledge.

The book is well produced and illustrated. Problems and selected references follow each chapter and the general index seems satisfactory.

H. KALMUS

Untaken Harvest. By GEORGE ORDISH. Pp. 171. London: Constable and Co. 1952. 15s.

The quotation on the flyleaf is felicitously chosen from *Cold Comfort Farm*. Should the author require another, he might select this from Bawden's *Plant Diseases*: 'It is often said that agriculture and horticulture afford a natural and healthy life. For the people engaged in them this may be true, but for the crops, nothing could be further from the truth.' Chapter and verse for this statement are amply provided in Mr Ordish's book.

Early in chapter 2, the author considers the British potato crop, now grown on approximately 1,100,000 acres, which could be produced on 106,000 fewer acres if a 10% loss due to disease were obviated. These 106,000 acres exemplify the instances assembled of land wasted by the 'untaken harvest'—the depredations due to plant pests and diseases.

Another equally valuable conception elaborated by Mr Ordish is the ratio of potential benefit to cost of control measures. In chapter 3 this is computed in detail for a series of treatments on apples, potatoes, wheat, turnips, sugar-cane and tea. The results are summarized in Table IX, which merits a most careful study by all those concerned in recommending pest and disease control measures.

The author frankly admits that the wide-ranging lists of losses given in Tables I-VIII are tentative and incomplete. In a useful, if cryptic, phrase these lists may be appraised as 'very good for what they are'; and Mr Ordish has earned our gratitude for bringing them together in a convenient compass. It appears that he does less than justice to his main contention in including 'Cost of control' under the general heading of 'Crop losses'. If these two concepts are to be equated it would seem necessary to set against the cost of applying control measures an estimate of the losses thus overcome.

Of the remaining chapters on methods of plant protection and on the social effects of crop losses, chapter 7 dealing with the trade in insecticides and fungicides is of particular interest as the first authoritative history of this trade in the United Kingdom. The final table provides statistics on world consumption, production, imports and exports of 'pesticides', of which British exports at £3,000,000 (1949 figures) compare reasonably with the U.S. exports of £10,000,000.

A book on crop losses could, understandably, be prosaic, but every page of *Untaken Harvest* is enriched by the breadth of perception of the author. Mr Ordish is at home in the potato field, the orchard, the library and, above all, in the vineyard: from these and many further sources he culls his evidence with fairness and discrimination. He has made a lively, gallant attack on a problem that has long been intractable and can never be inescapable.

R. W. MARSH

Manual of Bacterial Plant Pathogens. By CHARLOTTE ELLIOTT. Second, entirely revised edition (*Annales Cryptogamici et phytopathologici*, Vol. 10). Waltham, Mass.: The Chronica Botanica Co.; London: Wm. Dawson and Sons Ltd. \$6.00; 45s.

Some expository books may withstand the wear of time almost indefinitely. It is inevitable however that the informative kind, like this work under review, should sooner or later become outdated. The twenty-one years that have elapsed since the issue of the first edition of Charlotte Elliott's *Manual of Bacterial Plant Pathogens* (reviewed by R. H. Stoughton in *Ann. appl. Biol.* 17, 808) have seen a vigorous extension of all aspects of our knowledge of the plant pathogenic bacteria and the diseases they cause. Many new and important species have been described and many old ones reduced to synonyms, and for all pathogens, even the historic ones, a formidable amount of new information has accumulated. Most important however has been the rapid progress towards a stable and uniform system of nomenclature resulting from the wider and richer understanding of the natural affinities of these organisms.

With every advance the original edition of the *Manual of Bacterial Plant Pathogens* has become progressively outdated and the need for revision more and more apparent. This second revised edition will therefore be received by microbiologists with great satisfaction. It brings up to date all information on the subject and embodies the present ideas on classification and nomenclature. The system used is broadly that of Bergey's *Manual of Determinative Bacteriology* (1948) and is the one generally adopted by American phytopathologists. The Gram-positive pathogens are included in the genus *Corynebacterium* and the lophotrichous Gram-negative forms in either *Pseudomonas* or *Xanthomonas*. The peritrichous Gram-negative pathogens, on the natural affinities of which our ideas are less clearly crystallized, are divided amongst the two genera *Erwinia* and *Agrobacterium*. The generic term *Bacterium* is retained temporarily for a small group of unrelated species whose affinities still remain obscure. However critically this system may be regarded, it is unquestionably a considerable advance on E. F. Smith's modification of Migula's system used in the first edition, and represents for the first time a classification with something of permanency about it.

Apart from the obvious differences in the nomenclatural system, the pattern of the new edition closely follows that of the old. In Part I the bacteria are listed in alphabetical order together with their synonyms, chief morphological and cultural characteristics, a description of the disease symptoms they cause and a summary of the chief control measures. Under each species an extensive chronological list of references is given. Part II is a list of approximately 100 organisms associated with plant diseases but not recognized as valid plant pathogens. This time the author has wisely excluded the 'Chronological Chart of Plant Pathogens' (Part III of the first edition).

The bibliography, undoubtedly the most valuable feature of this work, is open to certain criticisms. It is exhaustive enough to intimidate any one approaching the literature of certain species for the first time, and, since the content of a paper is not always evident from its title, it is frequently difficult to distinguish the important references from the unimportant. The value of the bibliography would have been increased therefore if some discrimination had been made, possibly by short abstracts, between important references and those merely containing a passing mention of a particular organism. For a few species the reader is referred to the original edition for literature prior to 1930. This seems unnecessary as so little extra space would have been required to make this new edition completely independent of the old, with its out-dated and archaic nomenclature.

To have met these criticisms would have enlarged the scope and size of the book in its present form. By omitting the lists of specific cultural characteristics however, considerable space might have been made for these purposes without detracting much from the value of

the book. The purpose of these lists, which add little to those already given in Bergey's *Manual*, is not very clear. They are of necessity condensed, but at the same time they are needlessly repetitive for some generic characteristics. On the other hand, important data are omitted; thus under *Ps. mors-prunorum*, for example, wrongly described as Gram-positive, none of the distinctive characteristics useful in distinguishing it from the associated pathogen, *Ps. syringae* f.sp. *prunicola*, are given. Obviously these lists were never intended as a system of cultural identification (even where the available data for this are adequate), and anyone seriously interested in the characteristics of individual pathogens would be best advised to refer to the original papers.

Despite these criticisms, however, this new edition is a great improvement on the old. It has the considerable advantage of being based on a sounder and more enduring system of classification and nomenclature, and its intrinsic value is thus correspondingly enhanced. More discretion has been used in deciding which species should be included in the pathogenic and non-pathogenic lists. The method of indexing, by specific epithets with the generic names in parentheses, makes for easier reference, and will retain its value in the face of further changes in nomenclature since these are most likely to involve only generic names.

The author is to be congratulated on the publication of this new edition of the *Manual of Bacterial Plant Pathogens*, which remains, like its predecessor, an outstanding contribution to phytopathological literature. It will be of inestimable value as a standard work of reference to all interested in the phytopathogenic bacteria. Finally, the production maintains the familiar excellence of the *Chronica Botanica* series.

J. E. CROSSE

Probit Analysis: A Statistical Treatment of the Sigmoid Dosage Response Curve.

By D. J. FINNEY. With a foreword by F. TATTERSFIELD. Second edition.

Pp. xiv + 318. Cambridge University Press. 1952. 35s.

It is a pleasure, though no surprise, to see that a second edition of Finney's *Probit Analysis* has been called for. The soundness and value of the principles and methods so ably expounded in the first edition (see *Ann. appl. Biol.* **34**, 469) have become even more apparent in the five years which have elapsed since its appearance. The changes which the second edition records are therefore developments rather than amendments. Finney has found it necessary to rewrite only four of the forty-eight sections of which the first edition consisted, and even these, covering alternative types of response curves, the estimates of adjustments for natural mortality, the Parker-Rhodes equation and quantitative responses, are concerned with extensions of the probit method which can now be seen in a clearer light.

A new chapter has been added on 'Recent Developments', which, while necessarily somewhat 'bitty', will be widely welcomed. In particular, the further discussion of factorial experiments, the description of staircase estimation and the clarification of the meaning of standard errors, will do much to promote both the more efficient planning of experiments and the more confident interpretation of their results.

Certain of the tables which form so valuable a feature of the book have been extended, and two new tables added to aid in the use of new methods described in the additional chapter. Appendix II, which covers the mathematical derivation of the method, has been rewritten in a way which, while centring on the probit method, brings out more fully the principles underlying the treatment of response curves other than that from which probits spring.

Only one change is to be regretted, and that one which must have caused as much concern to the author as to his prospective reader. The book has been extended by rather less than a quarter of its original length, but the price has been nearly doubled. One can only accept this as another unfortunate sign of the times; and even now no one interested in the type of problem with which Finney deals need fear that his expenditure will be anything but fully repaid.

KENNETH MATHER